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THE COMPARATIVE EFFECTS OF PARATHYROID AND THYROID FEEDING ON GROWTH AND ORGAN HYPERTROPHY IN THE WHITE RAT[1]

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While thyroid feeding partially or completely inhibits growth in the young white rat (2) and produces marked hypertrophy of heart, liver, kidneys, spleen and lymph-glands (6), (5), (2) no organ-hypertrophy is produced by administration of thymus, pineal or pituitary (6), and adrenal only produces hypertrophy of the testes (7).

At the present time there is a tendency to consider the actions of thyroid and parathyroid as being opposite in nature (cf. for example, Langdon Brown (1)). We have therefore studied the effects of parathyroid tissue on growth and on hypertrophy of body organs in the growing rat, utilizing our previous results with thyroid for comparison.

Experimental results. White rats were used throughout the experiments. Their general treatment was similar to that described by us in previous papers. The diet consisted of unlimited bread and milk.

We have completed four series of experiments. In the first three desiccated beef parathyroid (obtained from the Armour Laboratories, and iodine-free) was fed daily in doses bearing fixed ratios to the actual body-weight of the animal under treatment, corresponding doses of desiccated beef liver being fed certain controls. In the final experiment a direct comparison of the effect of the parathyroid powder and of thyroid was made. The desiccated hog-thyroid used contained 0.34 per cent of iodine.

[1] Part of the expense of this research was defrayed by a grant from the Chemical Society (London), for which we desire to express our thanks.

In all cases the parathyroid powder was mixed to a paste with water and flour, and fed shortly after weighing the animal each morning, the amount always bearing a constant ratio to the actual weight of the animal at the time. At the end of each experiment the animals were killed and immediately dissected, and the organs transferred to closed glass vessels and weighed. The muscle used for comparison was the right tibialis anterior. The lymph glands were those from the anterior triangles of the neck.

TABLE 1

AGE	RAT 1, CONTROL	RAT 2, LIVER 1:500	RAT 3, PARATHYROID 1:5000	RAT 4, PARATHYROID 1:2000	RAT 5, PARATHYROID 1:500
days	*grams*	*grams*	*grams*	*grams*	*grams*
51	86	104.0	94.0	102.5	95
54	90	111.5	104.5	109.0	108
60	116	135.0	126.0	128.0	119
66	142	154.0	146.0	154.0	141
72	160	170.0	161.0	166.0	155
Weight increase in 18 days...	70	58.5	56.5	57.0	47
Percentage.....	78	52.0	54.0	52.0	44
Weight of organs					
Liver.....	9.6	10.9	8.8	9.8	9.9
Kidneys.....	1.44	1.40	1.30	1.45	1.15
Heart.....	0.64	0.65	0.57	0.66	0.69
Testes.....	2.15	2.21	2.09	2.25	2.21
Spleen.....	0.436	0.411	0.431	0.455	0.472
Adrenals.....	0.026	0.024	0.023	0.025	0.027
Thyroid.....	0.0100	0.0149	0.0112	0.0124	0.0111
Muscle.....	0.296	0.324	0.300	0.321	0.386
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Liver.....	6.0	6.4	5.5	5.9	6.4
Kidneys.....	0.90	0.82	0.81	0.88	0.97
Heart.....	0.40	0.38	0.35	0.40	0.45
Testes.....	1.34	1.30	1.30	1.36	1.42
Spleen.....	0.27	0.24	0.26	0.27	0.30
Adrenals.....	0.016	0.014	0.014	0.015	0.017
Thyroid.....	0.0062	0.0088	0.0069	0.0075	0.0072
Muscle.....	0.185	0.191	0.186	0.193	0.249

Experiment 1. Five males were used of a litter of seven rats born October 27 1920. Treatment commenced on the 54th day of age. Three rats were fed parathyroid in ratio of 1:500, 1:2000 and 1:5000 of body-weight respectively. A fourth was fed liver in the ratio of 1:500 of body-weight, while the fifth was fed bread and milk only. The rats were killed after 18 days' treatment. The figures for body- and organ-weights are shown in table 1.

Experiment 2. Five males were used of a litter of seven rats, born December 27, 1920. Feeding was commenced on the 46th day. Two rats were fed parathyroid in ratio of 1:1000, and one in ratio of 1:500. Liver was fed a fourth in the latter ratio, and the fifth received bread and milk only. The rats were killed after 36 days' treatment. The figures for body- and organ-weights are

TABLE 2

AGE	RAT 1, CONTROL	RAT 2, LIVER 1:500	RAT 3, PARATHYROID 1:1000	RAT 4, PARATHYROID 1:1000	RAT 5, PARATHYROID 1:500
days	*grams*	*grams*	*grams*	*grams*	*grams*
43	51	51	47	53	52
46	58	57	48	62	58
55	66	73	60	68	67
64	74	94	80	83	85
73	86	111	99	94	105
82	98	122	118	102	116
Weight increase					
In 18 days	16	37	32	21	27
In 36 days	40	65	70	41	58
Percentage	69	114	146	66	100
Weight of organs					
Liver	5.7	7.4	8.1	7.3	6.2
Kidneys	0.98	1.16	1.32	1.12	1.22
Heart	0.40	0.52	0.53	0.49	0.52
Testes	1.74	2.11	2.02	1.79	1.93
Spleen	0.250	0.254	0.377	0.238	0.319
Adrenals	0.019	0.025	0.020	0.019	0.020
Thyroid	0.0122	0.0152	0.0108	0.0105	0.0101
Muscle	0.199	0.245	0.218	0.184	0.218
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Liver	5.8	6.0	6.9	7.2	5.3
Kidneys	1.00	0.95	1.12	1.10	0.95
Heart	0.41	0.43	0.45	0.48	0.45
Testes	1.77	1.73	1.71	1.75	1.66
Spleen	0.25	0.21	0.32	0.23	0.27
Adrenals	0.019	0.020	0.017	0.019	0.017
Thyroid	0.0124	0.0124	0.0092	0.0103	0.0087
Muscle	0.203	0.201	0.185	0.180	0.188

given in table 2. Rat 3, treated with parathyroid, showed a similar development of pus in one of the lymph glands anterior to the thyroid cartilage to that noted previously by us in a rat fed thyroid (rat 1, exper. 6, Cameron and Carmichael (2)), and a similar condition has been noticed in a rat on a vitamin-deficient diet.

Experiment 3. Five females were taken of a litter born January 7, 1921. Feeding commenced on the 53rd day. Two animals were fed parathyroid in ratio of 1:1000 of body-weight, one was fed liver in ratio of 1:500, and two controls were fed bread and milk only. The rats were killed after 18 days' treatment. The figures for body- and organ-weights are given in table 3.

TABLE 3

AGE	RAT 1, CONTROL	RAT 2, CONTROL	RAT 3, LIVER 1:500	RAT 4, PARATHYROID 1:1000	RAT 5, PARATHYROID 1:1000
days	*grams*	*grams*	*grams*	*grams*	*grams*
50	71	79	78	83	81
53	76	88	84	87	84
59	86	98	97	96	96
65	101	107	111	112	113
71	110	125	122	127	130
Weight increase in 18 days...	36	37	38	40	46
Percentage.....	47	42	45	46	55
Weight of organs					
Liver.....	5.1	6.1	4.9	7.7	8.4
Kidneys.....	0.93	1.00	1.04	1.18	1.31
Heart.....	0.49	0.53	0.54	0.56	0.62
Spleen.....	0.277	0.301	0.266	0.334	0.351
Adrenals.....	0.025	0.028	0.023	0.028	0.033
Thyroid.....	0.0109	0.0115	0.0120	0.0126	0.0085
Muscle.....	0.202	0.222	0.209	0.220	0.234
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Liver.....	4.6	4.9	4.0	6.1	6.4
Kidneys.....	0.85	0.80	0.85	0.93	1.01
Heart.....	0.45	0.42	0.44	0.44	0.48
Spleen.....	0.25	0.25	0.22	0.26	0.27
Adrenals.....	0.023	0.022	0.019	0.022	0.025
Thyroid.....	0.0099	0.0092	0.0098	0.0099	0.0065
Muscle.....	0.184	0.178	0.171	0.173	0.180

Experiment 4. Six females were taken of a litter born February 1, 1921. Feeding was commenced on the 39th day. Two animals were fed parathyroid in ratio of 1:500, one was fed liver in the same ratio, two thyroid in ratio of 1:5000, and one control received bread and milk only. On the 10th day of treatment both thyroid-fed rats developed unmistakable tetany at approximately the same time (4 to 5 hours after thyroid had been fed, and before the daily meal of bread and milk had been given). All symptoms were present, flexion of fore-limbs, extension of hind-limbs, increased respiration and heart-beat, and the tetanic spasms. One animal died in 15 minutes, the other in 40 minutes after the tetany became distinct. These two rats, with the control fed bread and milk only,

were immediately dissected and weighed; the parathyroid-rats and liver-control were killed and weighed the following morning. The figures for body- and organ-weights are given in table 4. The period of treatment was too short to produce marked thyroid effects, except perhaps hypertrophy of heart and kidneys.

TABLE 4

AGE	RAT 1, CONTROL	RAT 2, LIVER 1:500	RAT 3, PARA-THYROID 1:500	RAT 4, PARA-THYROID 1:500	RAT 5, THYROID 1:5000	RAT 6, THYROID 1:5000
days	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
36	35	35.5	38	38	41	35
39	44	43.0	44	41	46	45
42	51	51.0	51	50	50	50
45	58	59.0	57	60	55	53
48	65	65.0	63	66	58	59
49	67	67.0	67	68	59	60
50		67.0	66	68		
Weight increase						
In 10 days	23				13	15
In 11 days		24	23	27		
Percentage	52	56	52	66	28	33
Weight of organs						
Liver	3.8	4.7	4.3	5.1	3.4	4.2
Kidneys	0.69	0.77	0.81	0.77	0.83	0.81
Heart	0.35	0.37	0.38	0.39	0.41	0.44
Spleen	0.173	0.215	0.203	0.233	0.170	0.199
Adrenals	0.014	0.016	0.016	0.019	0.015	0.017
Thyroid	0.0047	0.0068	0.0046	0.0047	0.0049	0.0052
Muscle	0.104	0.158	0.130	0.121	0.112	0.099
Lymph glands	0.056	0.064	0.060	0.073	0.072	0.052
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Liver	5.7	7.0	6.6	7.5	5.7	7.0
Kidneys	1.03	1.14	1.22	1.14	1.41	1.35
Heart	0.52	0.55	0.58	0.57	0.70	0.73
Spleen	0.25	0.32	0.31	0.34	0.29	0.33
Adrenals	0.021	0.024	0.024	0.028	0.025	0.028
Thyroid	0.0070	0.0101	0.0070	0.0069	0.0083	0.0087
Muscle	0.155	0.236	0.197	0.178	0.190	0.165
Lymph glands	0.083	0.095	0.091	0.109	0.122	0.087

DISCUSSION OF RESULTS

Experiment 1 appeared to indicate a possible decrease in growth-rate, from feeding parathyroid, unaccompanied however by any apparent hypertrophy exceptt hat of muscle. The remaining experi-

ments did not confirm such decrease. There were somewhat greater variations than usual in rate of growth, but no effect resembling that of thyroid fed in much smaller doses for similar periods. The very heavy doses of parathyroid and liver fed constituted a large part of the total food eaten, and thus much more varied diets were given these rats than in previous experiments in which the diet consisted almost entirely of bread and milk, with but small amounts of animal tissue.

Taken as a whole the results indicate that even very heavy doses of parathyroid produce no definite effect on growth, and no organ-hypertrophy. Comparatively heavy doses of liver are also without definite effect.

The thyroid glands were invariably normally red in color after parathyroid feeding, and there seemed to be no disappearance of fat.

The causes underlying the development of tetany in rats fed moderately heavy doses of thyroid will be dealt with more fully later. So far we have observed six definite cases of such tetany, two of which have already been reported (2). Age appears to be a marked factor in determining its production. The younger the animal when thyroid feeding commences, the greater the possibility that tetany may ensue. Such tetany cannot be considered as connected with parathyroid function and is almost certainly bound up with the increased respiration produced by thyroid administration which is a marked factor in such cases and which has been shown to induce tetany (3), (4).

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THE EFFECT OF THYROID FEEDING ON GROWTH AND ORGAN-HYPERTROPHY IN ADULT WHITE RATS

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In a recent paper by Cameron and Carmichael attention was drawn to the hypertrophy of lymphoid tissue following thyroxin administration to young rats, and the resemblance between this and the accompanying heart hypertrophy, and the observed hypertrophy of these tissues in fatal cases of hyperthyroidism (5).

We have attempted to ascertain whether these hypertrophies, and those produced in other organs by thyroxin or thyroid administration to young animals, are also produced in adult white rats.[1] Only one series of experiments was carried out, but the results were sufficiently concordant to warrant definite conclusions being drawn. Thyroid was fed, since Cameron and Carmichael have shown that, on basis of iodine content, more marked results are produced with thyroid than with thyroxin when fed by mouth. (Cf. also Plummer and Boothby (6).)

Experimental results. Seventeen rats, whose weights indicated that they were mostly young adults, were obtained from a single source, all being the inbred descendants of a single female. Of the eight females, six dropped litters during the preliminary observation period. Five of these females were rejected.

The rats were isolated in small cages, kept side by side under the same room temperature conditions, and were given an unlimited diet of bread and milk from January 10th to March 4th. Weekly weighings (to the nearest 0.5 gram) indicated that they had all ceased to show marked weight changes by the latter date. From March 4th thyroid (0.38 per cent iodine) weighed to the nearest milligram, and then mixed with flour and water to a thick paste, was fed five males and

[1] Some of the animals fed thyroid in Hoskins' experiments were autopsied at ages a little younger than those recorded in this paper, but had been fed thyroid from the age of 3 weeks. These of course all showed the organ hypertrophies.

two females daily for 18 days, in the ratio of 1:5000 of actual body-weight at the time of feeding. The thyroid-flour paste was given on a watch-glass immediately after weighing each animal, and was eaten completely. The thyroid used was a Merck (Darmstadt) preparation at least 10 years old, previously tested against a recent Armour hog-thyroid preparation, and found to give very similar results.

The remaining animals were kept as controls, and all animals continued to be fed unlimited bread and milk, the meal being given 3 or 4 hours after administration of thyroid to the treated animals. Cameron and Carmichael have shown that doses of desiccated liver tissue fed in the same ratio to body-weight as thyroid are without perceptible effect, so that in this experiment no such controls were used.

On March 22nd the rats were killed, and the body organs dissected, transferred immediately to closed glass vessels, and weighed.

All the macroscopically visible lymph glands in the anterior triangles along the internal jugular veins and above the level of the thyroid cartilage were dissected out and weighed together, constituting the "lymph glands" of the tables. The right anterior tibialis muscle was taken for comparison of muscle tissue.

The body-weights are shown in table 1, and those for various organs in table 2.

The thyroids of the treated rats appeared in all cases to be paler than normal. Lymph glands in the region of the axillae were distinctly enlarged. There was no complete disappearance of fat, but all the controls showed distinctly more fat.

DISCUSSION OF RESULTS

The figures in table 1 suggest that even in adult rats administration of thyroid produces a distinct retardation of growth. While the treated rats show a loss of weight, the controls still show a slight gain. Donaldson's tables (2) show that growth can continue to the 500th day of age, i.e., during half the maximum age of the animal. It is doubtful whether the loss of fat and other tissue is sufficient to account for the actual loss of weight observed plus the gain which would have occurred had thyroid not been fed.

The figures in table 2 show an actual gain of weight in liver, kidneys, heart, spleen, lymph glands and adrenals, while the thyroids average a smaller weight. The difference in the body-weights prevents a

TABLE I

DATE	MALE									FEMALE		
	Thyroid-fed					Control				Thyroid-fed		Control
	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Rat 7	Rat 8	Rat 9	Rat 10	Rat 11	Rat 12
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
January 10	207.0	209.0	286.0	213.0	162.0	209.0	195.0	219.0	187.0	198.0	123.0	
January 17	212.0	214.5	280.0	221.0	173.0	220.0	218.0	225.0	190.0	201.0	238.0	200.0
January 26	230.5	228.0	295.0	234.5	196.5	228.0	230.0	238.0	215.0	217.0	150.0	205.0
February 4	236.5	228.5	292.5	244.0	206.0	244.5	237.0	250.0	224.5	213.0	149.0	219.5
February 11	241.0	245.5	302.5	257.5	208.0	243.5	244.5	256.0	231.0	217.0	150.5	213.0
February 18	242.5	257.0	308.5	264.0	223.0	249.5	246.0	256.5	235.5	221.5	155.0	212.0
February 25	251.5	254.5	316.5	260.0	225.0	254.0	257.0	262.0	243.0	224.5	160.0	213.5
March 4	251.5	253.0	310.5	258.0	232.5	254.5	255.5	261.5	243.5	222.0	163.0	216.0
March 7	248.5	246.5	307.0	254.0	228.0	260.5	261.0	271.0	251.5	211.0	162.5	219.0
March 10	243.5	245.5	304.5	247.5	226.0	266.0	266.0	271.5	249.5	207.5	158.0	219.0
March 13	234.5	232.5	297.5	238.5	218.5	264.0	264.0	270.0	251.0	205.0	158.0	216.0
March 16	237.5	234.0	297.0	237.0	224.0	267.0	271.0	281.0	259.0	207.0	157.5	217.0
March 20	218.0	221.0	277.5	226.0	218.5	256.5	274.5	281.5	268.5	208.0	158.0	221.0
March 22	228.5	229.5	285.5	234.0	230.5	255.0	278.0	289.0	271.0	210.0	163.0	224.0
Weight change	−23.0	−23.5	−25.0	−24.0	−2.0	+0.5	+22.5	+27.5	+27.5	−12.0	0	+8.0

TABLE 2

Weight of organs

	MALE									FEMALE		
	Thyroid-fed					Control				Thyroid-fed		Control
	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Rat 7	Rat 8	Rat 9	Rat 10	Rat 11	Rat 12
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
Liver	14.9	14.9	17.6	16.1	14.0	11.1	14.7	16.7	14.1	18.4	13.1	13.0
Kidneys	3.22	3.14	3.91	3.23	2.98	2.24	2.37	2.79	2.56	3.33	2.40	2.40
Heart	1.21	1.15	1.32	1.11	1.09	0.94	0.91	0.92	0.89	1.10	0.84	0.82
Spleen	0.599	0.574	0.923	0.624	0.577	0.461	0.485	0.503	0.520	0.706	0.538	0.398
Lymph glands	0.141	0.174	0.186	0.200	0.196	0.150	0.131	0.097	0.136	0.151	0.166	0.143
Adrenals	0.035	0.035	0.036	0.036	0.029	0.028	0.027	0.023	0.026	0.074	0.051	0.053
Thyroid:												
Fresh	0.0130	0.0125	0.0111	0.0133	0.0135	0.0171	0.0140	0.0189	0.0206	0.0151	0.0135	0.0161
Dry	0.0038	0.0040	0.0032	0.0040	0.0049	0.0043	0.0033	0.0047	0.0053	0.0040	0.0038	0.0044
Muscle	0.361	0.372	0.487	0.364	0.381	0.527	0.505	0.510	0.455	0.367	0.268	0.416
Length, mm.	215	215	210	215	215	224	225	225		210	190	215
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Liver	6.5	6.5	6.2	6.8	6.1	4.3	5.3	5.8	5.2	8.8	8.0	5.5
Kidneys	1.41	1.37	1.37	1.38	1.30	0.88	0.85	0.97	0.94	1.59	1.47	1.02
Heart	0.53	0.50	0.46	0.47	0.47	0.37	0.33	0.32	0.33	0.52	0.52	0.35
Spleen	0.26	0.25	0.32	0.27	0.25	0.18	0.17	0.17	0.19	0.34	0.33	0.17
Lymph glands	0.062	0.076	0.065	0.086	0.085	0.059	0.047	0.037	0.050	0.072	0.102	0.061
Adrenals	0.015	0.015	0.013	0.016	0.013	0.011	0.010	0.008	0.010	0.035	0.031	0.023
Thyroid:												
Fresh	0.0057	0.0054	0.0039	0.0057	0.0067	0.0067	0.0050	0.0066	0.0076	0.0072	0.0083	0.0069
Dry	0.0017	0.0017	0.0011	0.0017	0.0021	0.0017	0.0012	0.0016	0.0020	0.0019	0.0023	0.0019
Muscle	0.159	0.162	0.171	0.155	0.165	0.207	0.182	0.176	0.168	0.175	0.164	0.178
Water content of thyroid	71	68	71	70	68	75	76	75	74	74	72	73

rigid comparison being made. Nevertheless, while most of the body-weights of the treated animals were lower, the weights of kidneys, heart, spleen, lymph glands and adrenals were actually greater in the treated animals, and the percentage figures accentuate the difference.

Although the body-weight changes were only relatively small, it might appear that loss of other tissue would give a simulated hypertrophy of the organs just mentioned, so that at any rate the percentage figures might be considered misleading. The prolonged preliminary observations permit growth curves to be drawn which allow estimations of the maximum weights which would have been attained by the treated rats, had thyroid not been administered. Table 3 gives the percentage weights of organs, using these estimated maxima. It will be observed that the same organs, and also the liver, show distinct hypertrophy even when this extreme test is applied. The hypertrophy is therefore actual.

The figures showing percentage of water in fresh thyroid tissue indicate that the glands in the treated rats are more anemic, in accordance with the view that they are in a resting condition.

Our results for adult rats are therefore in agreement with those found for young animals by Hoskins (4), Herring (3), and Cameron and Carmichael (1). Distinct hypertrophy of heart, liver, kidneys, adrenals, spleen and lymph glands is produced by only 18 days treatment with thyroid containing 0.38 per cent iodine, fed in the ratio of 1:5000 of actual body-weight. The thyroid gland in these animals enters into a resting condition. Muscle tissue shows distinct wasting, and there is some disappearance of fat.

The resemblance between the condition of various organs in hyperthyroidism and after thyroid administration can now be more aptly emphasized. The definite hypertrophies of heart and lymph tissues, present in both conditions, emphasize the precise parallelism of the clinical manifestations after administration of heavy thyroid doses and in hyperthyroidism to a degree sufficient to warrant the statement that if more accurate post-mortem examination of hyperthyroid cases be made, hypertrophy of other organs would be disclosed, especially of kidneys and adrenals.

The resting condition of the thyroid gland in animals subjected to thyroid feeding suggests that under normal conditions the output of thyroxin is determined by some factor in the blood passing through the gland—perhaps the thyroxin content of the blood itself—and as long as this remains above a certain level the setting free of thyroxin —presumably by the breakdown of iodothyroglobulin—is halted.

TABLE 3

	THYROID-FED RATS						CONTROL RATS				
	Percentage weights of organs corrected to (estimated) maximum body weights						Percentage weights of organs				
	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Average	Rat 6	Rat 7	Rat 8	Rat 9	Average
Estimated maximum weight (grams)	(275)	(275)	(320)	(280)	(255)		255	278	289	271	
Liver	5.4	5.4	5.5	5.7	5.5	5.5	4.3	5.3	5.8	5.2	5.1
Kidneys	1.17	1.14	1.22	1.15	1.17	1.17	0.88	0.85	0.97	0.94	0.91
Heart	0.44	0.42	0.41	0.40	0.43	0.42	0.37	0.33	0.32	0.33	0.34
Spleen	0.22	0.21	0.29	0.22	0.23	0.23	0.18	0.17	0.17	0.19	0.18
Lymph-glands	0.51	0.63	0.58	0.71	0.77	0.64	0.59	0.47	0.37	0.50	0.48
Adrenals	0.013	0.013	0.011	0.013	0.011	0.012	0.011	0.010	0.008	0.010	0.010
Thyroid:											
Fresh	0.0047	0.0045	0.0035	0.0047	0.0061	0.0047	0.0067	0.0050	0.0066	0.0076	0.0065
Dry	0.0014	0.0014	0.0010	0.0014	0.0019	0.0014	0.0017	0.0012	0.0016	0.0020	0.0016
Muscle	0.131	0.135	0.152	0.130	0.150	0.140	0.207	0.182	0.176	0.168	0.183

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FAT SOLUBLE VITAMINE[1]

IX. The Incidence of an Ophthalmic Reaction in Dogs Fed a Fat Soluble Vitamine Deficient Diet

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In the continuation of our studies on the factors operative in the maintenance of normal calcium assimilation (1), (2), we have had occasion to feed a group of dogs on a number of rations in which the vitamine content was varied within wide limits. As these rations constituted only a part of a series on the effects of which it is planned to report in detail later, only those facts which are considered relevant to the production of the ophthalmia, as suggested by experimental work on other animals, will be presented in this communication.

In the group of dogs under consideration, all were from the same litter and all were fed a daily basal ration consisting of 200 cc. centrifuged milk—heated for 1 hour at 15 pounds pressure—5 grams of precipitated calcium phosphate, 2 grams of sodium chloride, 5 grams of casein and a mash composed of equal parts of rolled oats and white cornmeal ad libitum. The latter was prepared by cooking in a pressure cooker at 15 pounds pressure for 1 hour.

Dogs 1, 2 and 3 received the basal ration without any supplementary additions. Dogs 4 and 5 received it supplemented with 20 grams of fresh cabbage daily and dogs 6 and 7 with 5 cc. of cod liver oil for the for the first 17 days and 10 cc. thereafter. Consumption of the desired ingredients was always secured by mixing them with a small amount of the mash. When this had been consumed, additional portions of the mash were given with water as found necessary. No digestive disturbances were ever noted.

Dogs 6 and 7 receiving the supplement of cod liver oil have maintained themselves in excellent nutritive condition. The former has

[1] Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

increased its weight from 2348 to 8270 grams and the latter from 1795 to 6510 grams in the course of 14 weeks.

Dogs 1 to 5 inclusive also increased in weight rapidly for the first 6 to 7 weeks, but even at the end of 5 weeks all gave signs of impending nutritive failure. In some cases there were indications of slight failure of appetite. Others were unusually quiet and walked with a peculiar gait and at times had difficulty in getting up or in standing on all fours. Sooner or later all developed deformities of the skeletal structures, as indicated externally or by radiograph examination. These abnormalities will be discussed in a later communication.

Dog 5 gave us the first indication that we possibly had reduced the fat soluble vitamine, even in its ration carrying 20 grams of fresh cabbage, to a level inconsistent with the maintenance of satisfactory nutrition. This was suggested by the fact that after 67 days on the ration its right eye became swollen and inflamed. Two days later the inflammation had spread, the purulency of the eye being reduced but the cornea having become leucomatous. Apparently the sight of this eye had been completely destroyed. Three days later the animal died. Inasmuch as this dog at the time of incidence of the ophthalmia was in a very poor nutritive state, not only from the standpoint of emaciation but also from the standpoint of extensive deformation of the thoracic cavity and cranium, no definite conclusions were arrived at as to the cause of the ophthalmia, as it was surmised that possibly the optic infection was due to a localized interference with normal nutrition brought about by pressure on trophic nerves.

Dog 4, on the same ration as dog 5, gave us a definite answer to this question. After 83 days on the ration, with more or less unsteadiness of gait being evident for 36 days of this time, but with no pronounced deformity, it developed a severe double ophthalmia. In the morning of the day of observation, the eyes were found to be merely dull in appearance and without expression, but in the afternoon both were filled with pus. Evidently a severe irritation had set in as the animal continually made attempts to rub its eyes with its paws or against the sides of the pen. On the following day the inflammation had attacked the cornea as indicated by the leucoma which was especially pronounced in the left eye. As the animal, though apparently very weak, was not severely emaciated—as a matter of fact it had not lost any weight whatsoever—an attempt was made to cure the ophthalmia by the administration of 20 cc. of cod liver oil in two portions daily. This was started on the evening of the second day when the ophthalmia

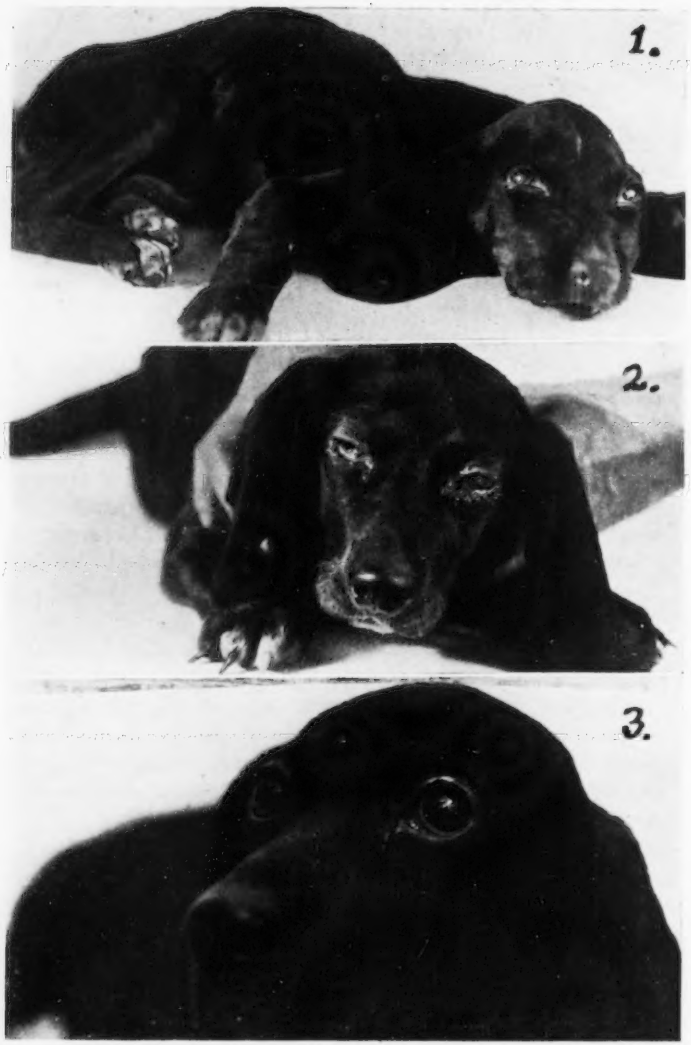

Fig. 1. Incidence of an ophthalmia in dog 5 after subsistence for 67 days on the basal ration consisting of 200 cc. autoclaved milk, 5 grams precipitated calcium phosphate, 2 grams sodium chloride, 5 grams casein and heated oatmeal and cornmeal ad libitum, supplemented with 20 grams of fresh cabbage daily. The right eye was severely infected, the left eye was apparently normal.

Fig. 2. A severe stage in the ophthalmic reaction observed in dog 4 on the same ration as dog 5 for 84 days. The keratitis was especially severe in the left eye. The animal had collapsed almost completely as it was necessary to support his head to obtain the photograph.

Fig. 3. Dog 4 after having been treated for 10 days with 20 cc. of cod liver oil daily. Recovery was complete except for a small area in the left eye where ulceration had occurred. This required 16 more days of medication before complete repair had taken place.

was first observed. The following day the eyeswere not as purulent, but it became noticeable that the keratitis of the left eyeball had proceeded to the ulcerated stage. The dog gave no signs of being able to see with either of its eyes. After 50 cc. of cod liver oil had been given that is, on the third day of medication, both eyes were observed to be considerably improved as indicated by the reduction of the leucoma and by the partial restoration of sight. From that time on improvement was continuous until after 26 days of cod liver oil feeding no trace of the ulcer remained. At the time of writing the dog is in good nutritive condition and rapidly gaining in weight.

On the basal ration alone supplemented with neither cod liver oil nor cabbage, dogs 1 and 2 died without any signs of an ophthalmia. In the case of dog 1 death occurred very early after having been on the ration for only 69 days. As it had grown very rapidly with the production of pronounced deformities and a poorly calcified skeleton its perverted mineral metabolism no doubt was a contributory cause. With dog 2 the immediate cause of death was an acute pneumonic inflammation, probably similar to that observed in rats on a fat soluble vitamine poor diet. From this standpoint the inflammation of the lungs may be taken as rather suggestive as to the responsible causal factors involved.

Dog 3, however, developed an ophthalmia after 94 days on the ration. The incidence of the ophthalmia was first indicated by a slight conjunctivitis for which an examintion was made when it was observed that the animal made repeated attempts to scratch its right eye. On the day following this observation the right eyeball had become completely leucomatous. As we were by this time firmly convinced that we were dealing with a fat soluble vitamine deficiency as the causal agent in these ophthalmias, we decided to determine the curative effect of a fat soluble vitamine preparation obtained by saponification and subsequent extraction of cod liver oil. Though the feeding of such a preparation had been included in our original plan of investigation on the effect of the fat soluble vitamine on calcium metabolism, the time now seemed opportune to determine the effect of such preparations on these ophthalmias as we have previously used them in our studies with rats (3).

For this purpose 500 grams of cod liver oil were saponified in two portions under a reflux condenser, each by boiling with 500 cc. of 20 per cent alcoholic potash for one-half hour. After cooling and diluting with 3500 cc. of water they were extracted thrice with generous por-

tions of ether. The extracts were united, brought down to dryness and taken up with alcohol, 50 cc. of 20 per cent alcoholic potash added (giving a concentration of approximately 4 per cent potassium hydroxide) and the solution was again boiled for one-half hour to insure complete saponification. At the end of this time the solution was again diluted with water and throughly extracted with ether. The ether extract was washed with water and then evaporated on a mixture of equal parts of cooked and dried cornmeal and rolled oats. Final weight of the preparation was made up to 500 grams with additional amounts of cornmeal and rolled oats, so that 1 gram of the preparation was equivalent to 1 gram of the original cod liver oil. During all these operations the material was guarded from the influence of light as much as possible as it is now well known that the fat soluble vitamine is very labile to the action of light.

Fifteen grams of the above preparation were given to the dog by forced feeding on the morning of the second day when the eye symptoms were first observed. In the afternoon of the same day the inflammation had spread to the left eye as indicated by a slight opacity of the cornea. A second administration of 15 grams of the preparation was given to insure a plentiful supply of the vitamine in the daily ration. On the following days medication with 30 grams of the preparation was continued. The appetite of the animal for the ration was slowly regained after the first day so that forced feeding was no longer found necessary. After 4 days of treatment the dog became much more active and the leucoma had decreased. After 2 more days both eyes were entirely normal and improvement in the general condition of the animal continued until at the time of writing an increase in weight of 480 grams in one week was observed.

SUMMARY

Within a period of 94 days three out of five dogs on a fat soluble vitamine poor diet came down with an ophthalmia such as has been observed by others in experiments with rats (4), mice (5), rabbits (6), chickens (7), and clinically also in man (8), (9).

Two dogs given a plentiful supply of this vitamine in the form of cod liver oil have remained entirely normal up to the present time of writing, which includes a period of 14 weeks.

Of the affected animals, one died shortly after the incidence of the ophthalmia. The other two were completely cured, one by the daily administration of 20 cc. of cod liver oil and the other by the administration of an ether extract of 30 grams of saponified cod liver oil.

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THE PULMONARY CIRCULATION TIME, THE QUANTITY OF BLOOD IN THE LUNGS AND THE OUTPUT OF THE HEART

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If V is the minute volume of the heart, Q the volume of blood in the lungs and T the mean pulmonary circulation time (in seconds), then $V = Q\frac{60}{T}$. When two of these quantities are known the third can be calculated (1). So far as I am aware, the three quantities have never been measured in one and the same animal. But V and T have been simultaneously measured by me (2), in anesthetized dogs, with unopened chest and abdomen, and V and Q by Kuno (3) on the heart-lung preparation, V being made to vary within wide limits in Kuno's experiments, principally by changing the venous pressure while the arterial pressure varied little. V alone has been measured by a number of observers in different animals and in man, and by different methods. Q alone has been estimated by Heger and Spehl (4), by Spehl (5) and by Spehl and Desguin (6), in rabbits; by Plumier (7) in dogs. Menicanti's observations (8) on Q in the dog, cat and rabbit are probably the least valuable, as he opened the thorax and rapidly tied off the lungs, without permitting them to recover their normal position and volume after adjustment of the ligature, as Plumier did in his careful experiments. On the whole, it may be said that the data in regard to Q are scantier and probably less satisfactory than those in regard to V and T. Observations on T alone have been made by me on many rabbits (9), a fairly large number of dogs, a good many cats and one bullfrog. Edmunds (10) has employed the same method mainly in rabbits (and some dogs) in a research on the influence of certain drugs, including adrenalin. Plumier (7), Steinhaus (11) and Langlois and Desbouis (12) also used my method in dogs, the last named observers for the purpose of investigating the effect of

asphyxia and adrenalin. All of these workers, except Edmunds, opened the carotid and inserted a tube containing electrodes which dipped directly into the blood. This entails the injection of an anticoagulant (Witte's peptone was used), and I cannot admit that the modification is at all an improvement on the original method, one of the advantages of which is that the artery does not need to be opened. The insertion of electrodes directly into the blood stream was carefully considered when the method was being worked out, and was rejected.

All my estimations were made more than 25 years ago but only those on the rabbits were published in detail. The chief reason for the delay in the publication of the data on dogs was, that by using animals of very different body-weights I had hoped to verify a relationship, tentatively suggested in a preliminary note (13), between T and the surface area. It seemed probable, accepting Rubner's law of the relation of the intensity of metabolism in homoiothermal animals to the surface, that V would also be some function of the surface. In a series of dogs going down to quite small body-weights V seemed to increase in the larger animals less rapidly than the body-weight, and rather in proportion to the surface. As might be expected, this appears more clearly when quite large and quite small animals are compared than in the middle range. It was hoped to obtain more data on this point also. Then if any fairly definite relation seemed to be established between V and surface, linear dimensions or body-weight, it was planned to make a series of determinations of Q in dogs of different size to see how closely the numbers actually obtained agreed with those calculated from V and T.

For example, it was conceivable that T might be approximately the same in dogs of any size. In this case $V = Q\frac{60}{T} = kQ$, where k is a constant. If now V varies as the body-weight or as a power of the body-weight, Q must also vary as the weight or as the same power of the weight. Thus, if $V = aW^n$, $Q = k'W^n$, where k' is a constant. If $n = \frac{2}{3}$, i.e., if V varies as the surface, Q must also vary as the surface.

But it is certain that whatever the relation between T and the body-weight or the surface may be, it is not, when measured under conditions as nearly uniform as possible, the same in a small as in a large dog. It does increase with the size of the animal but much more slowly than the body-weight, and even more slowly than the surface. In the preliminary note (13) I concluded, as a rough approximation, that if the animal is supposed to be rolled up into a spherical ball, T will vary as the diameter of the sphere, i.e., as the square root of the surface of the sphere. For animals of similar shape (dogs of different size) the same relation will hold good, only the constant being determined on the basis of

the "spherical animal." If in the equation $V = Q\frac{60}{T}$ we substitute this value for T, we get $V = k\frac{Q}{\sqrt{S}}$. From this it follows that if $V \propto S$, $Q \propto S^{\frac{1}{2}}$, or $Q \propto W$, where W is the body-weight. Probably this conclusion agrees with the preconceived idea of most physiologists, that the average quantity of blood in the lungs is approximately the same fraction of the body-weight in a large as in a small dog, and the same fraction of the total blood. For those, however, who consider that the total blood is more nearly related to the surface than to the body-weight, it may seem more probable that the quantity of blood in the lungs varies as the surface. In this case $V = k\frac{S}{\sqrt{S}} = k\sqrt{S}$, that is, the minute volume must then vary as the square root of the surface. This is easily shown to be wrong from available data on the output, which certainly increases in passing from the smaller to the larger animals of a series at least as fast as the surface. In the equation $V = aS^n$, if there is such a relation between V and S, the smallest possible value of n which will fit fairly ascertained facts is unity. Take now the supposition that V varies as the body-weight. Then we have $V = \frac{Q}{\sqrt{S}}k' = W$, from which $Q = k''W.W^{\frac{1}{2}}$, i.e., $Q \propto W^{\frac{1}{2}}$. In this case Q must increase more rapidly than the body-weight as we pass from the lighter to the heavier dogs. As this is certainly not the case for the lung weights, but if anything the opposite, it is not likely to be correct. Therefore, it is unlikely, in advance of measurements of V, that V varies as the body-weight and more likely that it varies as the surface. This is on the assumption that T varies as the square root of the surface and that Q probably varies as the body-weight.

If T varies as a lower power of S than the square root, say $T = aS^{\frac{1}{4}}$ and $Q \propto W$, we get $V = kW^{\frac{3}{4}}$, the minute volume increasing more rapidly than the surface but not as rapidly as the body-weight in passing from the smaller to the larger dogs.

While such calculations have a certain value even if one of the quantities can be shown to have a fairly definite relation to the body-weight or surface, their utility and interest would be much enhanced if such relationships could be established for all or at least for two of them. At the time of my earlier experiments I had hoped to extend them with the aim of obtaining more and possibly better data, particularly with reference to V and Q. Further reflection convinced me, however, that having regard to the circumstance that all the observations would have to be made on anesthetized animals, in which the quantities in question would necessarily vary from time to time in the same animal and from one animal to another, independently of any relation which might exist between them and the surface or body-weight, the number of experiments required to establish satisfactorily any definite relationships, assuming that such exist, would be very great. Quantities of

this kind, it is to be supposed, are more variable and above all more susceptible to the necessary experimental conditions than such a quantity as the total volume of blood in an animal (14), or the weight of a given blood-free organ, or the cross section of the aorta or trachea (15).

In the meantime also objections to Rubner's law became more numerous, and it seemed at any rate to need restatement and modification, as well pointed out by Benedict (16), although Du Bois (17) considers that when properly interpreted it is still in the main consistent with the results obtained for basal metabolism in man. More important for our purpose is the fact recently recorded by Barcroft and his collaborators (18), that some individuals of a species (goats) habitually utilize in the tissues a greater proportion of the oxygen taken up by the blood in passing through the lungs than others, so that a smaller minute volume of blood suffices to maintain the same intensity of metabolism. This must be coupled either with a smaller average linear velocity of the blood (corresponding to a greater average circulation time), if the total quantity of the blood is not lessened, or with a smaller total quantity of blood if the average linear velocity is not lessened (i.e., the average circulation time not increased). Theoretically it is obvious that there are other combinations which will cause a reduced minute volume. For instance, the quantity of blood might be increased but the average circulation time increased still more, or the quantity of blood might be diminished but the average circulation time not so much diminished. The quantity of blood in this connection is that which is effectively circulating at any time, not including any portion which may be more or less stagnant. It is clear that if marked habitual differences in the oxygen use can occur in different individuals, this would obscure any general relation of minute volume to surface, if this existed in the majority of the individuals of a series, at least until the size of the animals became so small that an increased utilization of oxygen-carrying capacity of the blood could no longer keep pace with the increasing intensity of the metabolism. For this reason I see no prospect of being able to carry the investigation farther, and desire now to publish the data accumulated, especially those on the pulmonary circulation time in dogs, which, I hope, possess some value in themselves, apart from the object for which they were originally obtained. In the animals in which T was directly determined as well as V, the question whether V is a function of the surface does not, of course, arise, and it becomes of interest to know what values are obtained for Q when the observed values are substituted for V and T.

Now that Barcroft and his pupils have modified the method of Zuntz (19), depending on comparison of the oxygen content of the mixed venous blood and of the arterial blood, and have shown that it gives remarkably concordant results for V in animals under constant anesthesia (20), there can be little doubt, I think, that it is the best method, for most purposes at least. The automatic passage into the blood of the substance (oxygen), the quantitative changes in which serve to measure the minute volume, is an advantage not enjoyed by a method depending on the injection of a salt. But probably the greatest advantage, when numerous estimations are to be made at short intervals, is that no water is introduced into the blood.

V and T are known to vary considerably in one and the same animal under different conditions. Unless V always varies inversely as T, Q must also vary. It has been shown that V does not always vary inversely as T. Although an increase in V is often associated with a decrease in T, it may be associated also with an increase in Q without change in T. It is possible that sometimes an increase in V may be accompanied both by a decrease in T and by an increase in Q, as illustrated by Kuno (3) on the heart-lung preparation, or even by an increase in T while Q increases still more. The actual occurrence of the last combination has not been sufficiently demonstrated.

In a separate paper will be given the weights of the lungs, heart and other organs of a series of dogs of widely varying body-weight, largely freed from blood by exhaustive bleeding. In these animals the area of the skin was also approximately measured, and certain interesting relationships of the organ weights to body-weight and surface seem to come out. In a number of additional dogs the weight of the lungs after deducting the residual blood contained in them (estimated colorimetrically) was obtained. Since the lung parenchyma may be looked upon as a very extensive thin membrane covered over the greater part of its surface with capillaries which do not differ materially in caliber, at least in animals of the same kind, the weight of the blood-free lung, freed from large bronchi, is probably pretty closely proportional to its surface, and therefore to its vascular capacity with the capillaries distended to average width under average pulmonary blood pressure. Kuno (3) showed on the heart-lung preparation that the quantity of blood contained in the two lungs of the same dog, which, as is well known, differ markedly in weight, the right being the heavier, is approximately proportional to the weight of the blood-free lungs. Weighing the lungs cannot, of course, take the place of proper determinations

of Q but is far easier to carry out, and, what is more important, is far easier to carry out correctly. And the relation of the lung weights to body-weight or surface might at any rate give some indication how Q might be expected to vary in a series of animals differing greatly in size, no good determinations of this kind having yet been made.

The time of the lesser circulation (in dogs). T is practically always obtained in the form of a crude number (time from a vein, usually the external jugular, to a systemic artery, usually the carotid). A correction must therefore be applied for the time from the left ventricle to the artery. This correction is as small as possible, in determinations made without opening the chest, when the point at which the arrival of the salt or pigment is noted is on the carotid low in the neck. It is less in small than in large animsls, and therefore in rabbits than in dogs. The "lost time" between the point of injection and the heart is usually negligible, since in my observations salt or methylene blue solution, injected from a syringe, or better from a burette, through a cannula in the jugular low in the neck or through a catheter passing into the superior cava, would reach the heart practically as soon as injection began. The cannula or catheter and probably the dead end of the vein are already filled with the solution. It is not at all advisable to inject into a distant vein like the saphenous, as was done by Plumier (7) in some of his experiments, since this entails a considerable correction, which cannot be accurately made merely by calculating from the average velocities in the veins given in the textbooks.

Another correction, which can only be roughly applied but which is unimportant when the heart is beating at a fairly rapid rate, concerns the time lost in the heart according to the point in the cardiac cycle at which the first of the solution enters the right ventricle. If it enters it just before closure of the tricuspid valve, with a pulse rate of 120 per minute, some of the salt will have passed into the pulmonary artery a small fraction of a second thereafter. But if the salt begins to enter the right ventricle immediately after the opening of the tricuspid, nearly half a second may elapse before it begins to pass into the lungs. If having begun to enter the right ventricle just after systole, the first of the salt happened to reach the left ventricle just after systole, the lost time in the heart might not be far from a second, but this would be the maximum possible. The average would be less than half a second. If the heart rate is only 60 a minute, as in a good many of our morphinized dogs, the average would be less than a second, and when the mean of a number of successive determinations of T is taken, the error is

automatically approximated to the average. If this correction is made, Q must be taken as the quantity of blood in the lungs, and T as the actual average time of transit of Q cc. of blood from pulmonary artery to pulmonary veins. But it is more convenient in general to consider T as the time from the entrance of the salt into the right ventricle to its appearance in the aorta. In this case Q must be taken as the blood in the lungs, plus a proportion of the blood in the heart varying from approximately twice the output of one ventricle per beat to nothing, according to the phase of the cardiac revolution at which the salt enters the ventricles. The average amount of blood to be added to that in the lungs will be equal to the output of one ventricle per beat.

Plumier (7) is not quite accurate in saying that all the blood in the heart (both sides) and lungs must be renewed in the time of the lesser circulation. This could only be true if the heart was not beating, and the ventricles were sitting full of blood while an artificial flow was kept up through the lesser circulation. This is easily seen if we suppose the lungs to contain blood equal to the amount ejected by a ventricle in 6 beats. If the first of the salt solution enters the right ventricle just before systole, then in practically the time of 5 beats it will have reached the left ventricle, the blood in the lungs at the time of arrival of the salt at the right ventricle having been displaced. If the salt happens to reach the left ventricle just before systole, it will be entering the aorta in little more than the time needed for 6 beats. In that case the time from right ventricle to aorta would correspond to scarcely more than the time needed to displace the blood in the lungs. If the salt entered both ventricles just at the end of systole, the time would be nearly that of 8 beats, and would correspond to the displacement of the blood in the lungs *plus* twice the blood thrown out by a ventricle per beat. On the average, then, for the lesser circulation Q may be taken as the quantity of blood in the lungs *plus* the output of one ventricle per beat, and Q will be displaced in T seconds, where T is the time from right ventricle to aorta. It is particularly in the case of small animals, with slow heart rate, that the "lost time" in the heart may cause a sensible error. In rabbits the pulse rate is generally so great that, for the degree of accuracy attainable in such measurements, the correction may be neglected. The same is true in large dogs even if the pulse is rather slow, because the circulation time is itself relatively long.

It will be observed that in discussing possible corrections to be applied to the gross circulation time from jugular to carotid, in order to arrive at the net value of T, nothing has been said as to the necessity of adding something to the observed gross time to compensate for the greater speed of portions of the salt moving in the axial stream than the average speed. Tigerstedt (21) has brought forward certain observations of v. Kries (22) on the flow of liquids in capillary tubes, as evidence that all such methods must give too short a circulation time, more nearly half of the average than the average time. If this were true it would not preclude the use of these methods for comparative observations. But the

results obtained for the net value of T, on the assumption that they represented the average circulation time, would give much too high values for V, or much too low values for Q, when substituted in the equation $V = Q\frac{60}{T}$. We shall see later on that this is not the case, but that if we were to double T, V would come out impossibly low or Q impossibly high. Indeed, the striking thing is that in experiments in which V and T were both determined, the values calculated for Q on the assumption that T is the average circulation time are distinctly higher than those directly obtained by Plumier (7) and of the same order of magnitude as those obtained by Kuno (3) on the heart-lung preparation. They lend support indeed to the suggestion of the last named author, that the quantity of blood in the lungs has been underestimated by previous workers. And if we were to increase the values for Q, which would be necessary if there was anything in Tigerstedt's contention, the fraction of the total blood contained in the lesser circulation would be incredibly large. The reasons for believing that the conclusions of v. Kries on the flow in glass capillary tubes cannot be transferred to a vascular tract like the pulmonary path, in which the greater part of the time of passage is unquestionably spent in the capillaries, have been given in previous papers (2), (23). The essential one is that, in a branching and anastomosing system of capillaries filled with blood corpuscles, it is impossible that a given particle of salt solution, or rather of the mixture of plasma and salt solution, should continue moving in an axial stream, with the maximum velocity, for more that a small fraction of the total circulation time. The path must be an "out and in" one in the spaces between corpuscles which, if in the axial stream at one point, are apt to be shunted out of it at a bifurcation or at an angle of the network. The dimensions of the capillaries also are such that a given corpuscle must sometimes lag and sometimes hasten, as its movement of translation is more or less impeded by contact with the walls or with other corpuscles, or by its own rotational movements.

One of the most direct proofs of the truth of this reasoning is that the observed time of passage of the altered column of blood over an artery, when salt or pigment solution is infused into the jugular vein, is in general not much longer than the time for which the infusion is kept up. The excess in the time of passage may be relatively greater with a short period of injection than with a longer period because the "lost time" in the heart, depending on the phase of the cardiac cycle at which the solution enters the ventricles, will be on the average of the same absolute magnitude. In such observations the quantity of residual blood in the ventricles after systole will also have an influence upon the time of passage of the column, although not upon the circulation time as determined by the arrival of the first portion. The washing out process must tend to prolong the time of passage, and since the absolute amount of the "lag" caused in this way will be the same for a short as for a long period of injection, the relative prolongation will be much

greater with very short injection periods. Since in spite of the lag due to washing out, the time of passage with injection periods of moderate duration (not so long as to allow a round of the circulation to be completed) only slightly exceeds the time of injection, or even coincides with it in length, when the animal is still normal, it must be concluded that "hastening on" of the salt in the axial stream does not sensibly affect the circulation time of capillary tracts, as measured by these

TABLE 1

DOG	WEIGHT	INJECTED		TIME OF PASSAGE	DOG	WEIGHT	INJECTED		TIME OF PASSAGE
		Amount	Time				Amount	Time	
		cc.	*seconds*	*seconds*			*cc.*	*seconds*	*seconds*
X	12.3	36	15	15	XVII	18.2	14	15	15.5
XIII	27.9	28	15	19			11	15	18
		29	15	19			13	15	13
		35	18	27	XVIII	9.9	43	13	13
		34	18	27	XIX	12.8	22	10	12
XIV	32.3	50	12.5	13			22	10	10.4
		45	14	14	XX	10.3	17	8	8
		38	10	11.4			16	8	9.5
		39	10	13.5			18	10	10.5
XV	4.9	15	12	13.5			16	10	10.5
		7	5	7.3			42	10	14.5
XVI	11.8	27	8	9			32	10	14.2
		26	8	9			28	10	14
		18	7	10	XXI	15.0	25	10	10
		13	5	9			24	12.5	12.4
		7	15	15			20	12	11.8
		12	10	15	XXII	17.5	33	10	10.5
		11	12	12.2	XXIV	34.5	32	10	9
		8	10	10.2	VI	13.3	15	12	11
		8	10	11.2			21	14	11
		8	10	11			30	14	12
		12	10	12.5			25	15	15

methods. A typical experiment follows, and numerous additional results are given in table 1.

Dog (puppy); weight 2.5 kgm. Morphine and ether. A 2.5 per cent salt solution containing 0.125 gram anilin blue black in 100 cc. was first injected (into the jugular). This is not as good a pigment as methylene blue, if numerous injections are to be made, since it soon causes permanent coloration of the artery. Injected 6 cc. in 5 seconds in 3 observations; times of passage, 5.4, 5.1 and 6.0 seconds. Immediately thereafter, 2 observations with injection of 4 per cent

salt solution for 5 seconds gave with the telephone method 4.8 and 5.6 seconds respectively as the time of passage. When 8 cc. of 2 per cent salt solution was introduced, in 7 seconds, the times of passage (in two observations) were 8.8 and 8.3 seconds. With 3 cc. of 8 per cent salt solution injected in 7 seconds, the time of passage was 7 seconds. With 12 cc. of 2 per cent solution injected in 15 seconds the time of passage was 17.9 seconds. It must be noted that in 15 seconds there was time for some of the salt to have completed a round of the circulation, and to have reached the electrodes a second time. Later on, when the circulation time to the carotid had increased (to 7 seconds, a very long time for so small a dog) the time of passage, with injection of 4 per cent salt solution for 5 seconds, increased to 7 and 8.8 seconds in two observations, and to 9.2 seconds in one observation with injection of 2 per cent solution for 5 seconds. The pulse rate was 120 a minute. No pigment was injected after the first three observations. The walls of the stomach and intestines were found deeply tinged, also the urinary and gall bladders, but no pigment was visible in the urine or bile. The kidney on section showed distinct blue coloration in the boundary layer, papillary zone and pelvis, none in the cortex. At the time of the last observations when the circulation time had increased, there can scarcely be any doubt that a condition of plethora existed, due to the injection of liquid equal to one-third of the original volume of the blood, and of twice as much sodium chloride as the blood could have originally contained. The increase in the circulation time was probably associated with an increase in the quantity of blood in the lungs, over-filling of the right heart and inability of the right ventricle to empty itself as completely as before. The washing out of salt solution from the right heart and superior cava would then prolong the time of passage materially.

In the experiments in table 1 the electrodes were on the femoral artery except in dog XVI, where they were on the carotid, and in dog XVII, in which they were on the carotid in the first observation and on the brachial in the others. In dogs XXI and XXII the injecting cannula or catheter was in the left ventricle, in XXIV in the descending thoracic aorta; in XX it was in the left ventricle in the first 4 observations, in the last 3 in the jugular. In all the other dogs the cannula or catheter was in the jugular. The beginning and end of the sound are sharper with injection into the left ventricle than with injection into the jugular vein. It should be noted that some of the times of passage are somewhat longer than the true time, because one often waited a little too long in order to be sure that the sound had declined to the minimum. This was noted in the last three observations on dog XX. The purposely much greater volume of solution injected as compared with the first 4 observations on this dog would also tend to increase the time necessary to "wash out" the ventricle. In dog XIII the observations were made at the end of an experiment, during which numerous injections of 5 per cent salt solution had been made. Some degree of plethora was probably present, increasing the time of washing out of the heart. With

the long time of injection, especially in the two last observations, an appreciable part of the salt might have completed a round of the circulation and arrived a second time at the electrodes, thus prolonging the apparent time of passage. This possibility was illustrated in the second and fourth observations on dog VI, in which a distinct reinforcement of the sound was noticed 16 seconds after the minimum had been reached. In dog XVI the common result, that with injection of a relatively large amount of solution in a short time, the time of passage tends to be decidedly longer than the time of injection, is seen in one or two of the observations. This has already been explained as due to the relatively greater "lost time" in the heart, and the longer time needed for "washing out."

In calculating the net time of the lesser circulation, the only way of of being quite certain of the correction necessary for the time from left ventricle to artery is actually to determine that time by observations on the same animal with injection of salt into the left ventricle. When this is done, an artery even as distant as the femoral can be employed without appreciable disadvantage. This was the case in many of the experiments, because a branch of the femoral was in any case being used to collect blood for estimation of the output of the heart. All the dogs were anesthetized with morphine, with in addition ether or ACE mixture. Where the length of the dog is given, it was measured from the tip of the nose to the anus, with the animal stretched upon the board.

Dog XXII, 17.5 kgm. Length 106 cm. From origin of aorta to mid-point between electrodes on femoral artery, 49.5 cm. Injection at first through cannula in left ventricle, then through catheter with its orifice just inside the right auricle. Salt solution 1.5 per cent. Time from left ventricle to femoral, 5.7 seconds. Minute volume per kgm., 221 cc. Time from right auricle to femoral, 11.5 seconds. Minute volume per kgm., 214 cc. Another observation: time from auricle to femoral, 12.5 seconds, minute volume per kgm., 185 cc. The heart rate varied from 69 to 80 a minute throughout the experiment. The average output (for 11 observations) was 212 cc. per kgm. per minute, but for the last observations, which concern us most in the present connection, 194 cc. The corrected time from right ventricle to aorta is 6 seconds.

Taking the minute volume at 190 cc. per kgm. per minute and substituting in the equation $V = Q\,\frac{60}{T}$, we get $3300 = 10\,Q$, or $Q = 330$ cc. Deducting 50 cc. for the average output of the ventricle per beat, 280 cc. would represent the blood in the lungs. If the total blood be taken at $\frac{1}{13}$ of the body-weight, say 1300 cc., this would represent $\frac{1}{4.6}$ of the blood. It is obvious that if Tigerstedt's (21) contention was

correct, and a much higher value, say 12 seconds, were taken for the average T, the impossible proportion of nearly half of the total blood would have to be attributed to the lungs. Even if we assume that the minute volume was over-estimated and take it as 150 cc. per kgm. per minute, less than the average for our series of dogs, the fraction of the total blood in the lungs would still be $\frac{1}{6}$. There was no evidence of plethora caused by the injections, the output varying little from beginning to end of the experiment. If there is any question as to the net circulation time from right ventricle to aorta, it is whether it should not perhaps be made slightly shorter by allowing half a second or so for lost time in the heart, which would make the fraction of the total blood in the lungs, with minute volume 190 cc. per kgm., $\frac{1}{5}$ (and with minute volume 150 cc., about $\frac{1}{7}$).

Dog XX, 10.3 kgm. Length of dog 84 cm. Origin of aorta to electrodes on femoral artery, 47 cm. Salt solution (2 per cent) at first injected into left ventricle and then through catheter passed into jugular.

10:46 a.m. Left ventricle to femoral 6 seconds. Pulse 74.
11:00 a.m. Left ventricle to femoral 6.5 seconds. Pulse 72.
11:04 a.m. Left ventricle to femoral 7.5 seconds. Pulse 80.
11:11 a.m. Left ventricle to femoral 8.5 seconds. Pulse 72.
Average: Left ventricle to femoral 7.1 seconds
11:29 a.m. Jugular to femoral 14.5 seconds. Pulse 79.
11:36 a.m. Jugular to femoral 15.8 seconds. Pulse 80.
11:44 a.m. Jugular to femoral 15.0 seconds. Pulse 90.
Average: Left jugular to femoral 15.1 seconds

Average time from jugular to aorta, 8 seconds. Deducting 1 second for the passage of the blood from the catheter to the right ventricle and for the lost time in the heart, we get 7 seconds as the shortest average net time which can be accepted. The average minute volume was 230 cc. per kgm., one of the highest obtained in our series for a dog of this size. Even if it were assumed that it was over-estimated, and the average for the larger dogs (about 150 cc.) were taken, the equation would give $1500=\frac{60}{7}Q$, or $Q=175$ cc.

Deducting 20 cc. for the output of the ventricle per beat, we get 155 cc. as the blood in the lungs, equal to $\frac{1}{5}$ of the total blood. Again the question is not whether T has been taken too low, as Tigerstedt (21) would maintain, but whether another second or so could not possibly be deducted from it. Even if this were done, and there is probably no justification for it, the fraction of the total blood contained in the lungs would still be little less than $\frac{1}{6}$. The output did not alter essentially throughout the experiment, and there is no evidence of plethora.

Of course, as in all these experiments after repeated injections of salt solution and removal of samples of blood, there was some diminution in the viscosity of the blood. The influence of this upon the minute volume is unknown.

Dog XXIII, 7.16 kgm. Length, 73 cm. Distance from origin of aorta to electrodes on femoral artery, 33 cm. Salt solution (1.5 per cent) at first injected through cannula in left ventricle, and then through catheter in jugular vein. In the first part of the experiment, from 10:40 a.m. to 11:30 a.m., when the heart was in good condition although beating slowly (from 37 to 52 a minute), the average of several observations on the time from left ventricle to femoral was 6.5 seconds, the average from jugular catheter to femoral, 11.5 seconds. Net time from right heart to aorta, 5 seconds. Average minute volume, 210 cc. per kgm. In the last part of the experiment, from 11:35 a.m. to 12:15 p.m., the average time from ventricle to femoral was 10 seconds, from right heart to femoral, 15 seconds (average of 4 observations, 14.5, 15.6, 14.9 and 14.8 seconds). Net time from right heart to aorta, 5 seconds. Pulse rate, 52 to 56. Minute volume in the last observation, 133 cc. per kgm. per minute, and 170 cc. per kgm. per minute at the beginning of this part of the experiment. The specific gravity of the blood at the beginning of the experiment was 1054.9; at 11:15 a.m., 1050.0. When repeated observations on the output are to be made by this method on the same animal, care should be taken to limit the volume of the injected liquid and of the blood withdrawn to what will suffice for satisfactory determinations. Where the results vary much in the course of an experiment, observations made early in the experiment are to be preferred.

Taking the average minute volume for the first part of the experiment at the round number 200 cc., we have $1400 = 12Q$, or $Q = 110$ (say). Deducting 30 cc. for the output per beat of the ventricle (taking the average pulse rate at 45), we get 80 cc. as the, blood in the lungs, or about $\frac{1}{7}$ of the total blood. For the second part of the experiment, with an average minute volume of 150 cc. per kgm., $Q = 85$ cc.; or deducting the average output of the ventricle per beat (20 cc.), the blood in lungs is 65 cc., about $\frac{1}{9}$ of the total blood.

It will be observed that in the above experiments the average time, over the whole of the path from left ventricle to femoral, is distinctly longer than would have been obtained if the measured distances were divided by conventional values for the linear velocity of the blood in the large arteries. This is illustrated in table 2, some of the data in which have been published previously (24).

The numbers in the last 4 columns are averages of a number of observations. For instance, in dog XXIV the following values were obtained for the time from the point of injection to the femoral at different times throughout the experiment: 4.5 seconds (4:18 p.m.), 4.0 seconds (4:23 p.m.), 4.8 seconds (4:38 p.m.),

4.8 seconds (4:46 p.m.) 4.9 seconds (5:35 p.m.). In dog XXI, 4.6 seconds (3:55 p.m.), 5.2 seconds (4:20 p.m.), 5.2 seconds (5:54 p.m.), 4.8 seconds (6:05 p.m.). Salt solution was injected into the left ventricle in all the numbered dogs except XXIV, in which the opening of the catheter was in the descending thoracic aorta. In dog XXIII it is noted that at the time of these observations the heart was weak. In dog XXV the chest was opened and the salt solution injected through

TABLE 2

DOG	WEIGHT	LENGTH OF DOG	DISTANCE TRAVERSED	TIME	PULSE RATE PER MINUTE	VELOCITY PER SECOND	DISTANCE PER HEART BEAT
		cm.	*mm.*	*seconds*		*mm.*	*mm.*
XXIV	34.5	121	420	4.6	105	91	52
XXII	17.5	106	495	5.7	69	87	75
XXI	15.0	92	400	5.0	102	80	47
XX	10.3	84	470	7.1	74	73	59
XXV	8.0	70	350	10.0	130	35	16
XXIII	7.2	73	330	7.8	46	42	55
	6.5		250	3.0		83	
	13.3		320	3.3		97	

a needle into the left ventricle. The heart was unaffected by the introduction of the needle, but the blood pressure was already low. The minute volume was the smallest measured in my experiments, 52 cc. per kgm. per minute, by procedure I (23), and 56 cc. per kgm. per minute in one observation by procedure II. The total quantity of salt solution (4.7 per cent) injected during the experiment was 15 cc., the total quantity of blood drawn, 35 cc. The length of the dog was 70 cm. In the last two dogs in the table (not numbered) the solution was injected into the carotid and detected in the splenic artery.

In another dog (C) weighing 9.8 kgm., the average time from a catheter in the jugular to the carotid low in the neck (7 cm. from the heart) was 9.2 seconds. The time from the jugular to the femoral artery (42 cm. from the heart, just below Poupart's ligament), was 15.2 seconds. To traverse the last 35 cm. of the arterial path to the electrodes on the femoral, 6 seconds was required, an average linear velocity of scarcely 60 mm. per second, or with an average pulse rate of 57 per minute about 63 mm. per heart beat. The (corrected) average time of the lesser circulation was reckoned at 7.5 seconds in this part of the experiment.

Later on pulmonary edema developed, with marked hyperpnea. The circulation time from the catheter in the jugular to the femoral artery was distinctly shortened, to 9.1 seconds (average of 6 observations) and the pulse rate much increased, first to 80 and at last to 146 a minute. The time of the lesser circulation must have been considera-

bly diminished, and this must have been associated either with an increase in V owing to the increased venous return due to the hyperpnea, or to a diminution in Q owing to a diminished vascular capacity of the lungs caused by the edema, while the right heart was still able to force through the lungs the blood which reached it.

Kuno (3) in two experiments on the heart-lung preparation, in which edema of the lungs was caused by the use of old blood for perfusion, found an increase in Q. He purposely kept the venous supply to the heart very small in order to imitate the pulmonary circulation in patients with edema of the lungs, which he assumes must be very slow. It is questionable how far such experiments can be transferred to the intact circulation. In any case no great stress should be laid on my single experiment, although it is an instance in which during edema the time of the lesser circulation was lessened.

The opposite condition is illustrated in the effect of asphyxia in augmenting the time of the lesser circulation. It was observed in my first series of experiments on rabbits (9) that a "venous" condition of the blood was associated with a lengthening of the pulmonary circulation time. When asphyxia was prolonged the increase was very marked in both rabbits and dogs. Later, this was observed by Langlois and Desbouis (12), and the amount of retardation measured systematically in a series of dogs. I do not intend to discuss here the cause of the lengthened pulmonary circulation time in asphyxia, further than to point out that changes in the systemic circulation (vasoconstriction with increased arterial pressure) and changes in the heart beat (stimulation of the cardio-inhibitory center, or weakening of the contraction, with engorgement of the right heart in prolonged asphyxia) must react upon the pulmonary circulation time, apart from any possible vasoconstriction in the lungs. The following experiment illustrates another condition (although here there may also be an asphyxial factor) in which the time of the lesser circulation has been found to be affected.

Dog. Male. 13.3 kgm. The animal had been used for a class demonstration of the cortical motor areas under ether. In exposing the brain some blood had been lost. Morphine (0.16 gm.) was now given and circulation time measurements made on various vascular paths.

1. *Injection of salt solution into left external jugular.* Time to right carotid, 6.3, 9.4, 7.1, 8.9, 7.2, 8.2, 8.0, 7.6 and 7.9 seconds in successive observations (average 7.8 seconds). Time from jugular to spermatic vein in the cord, 12.2, 14.7, 12.2, 11.5 seconds (average 12.6 seconds). The spermatic cord was placed on the electrodes. The galvanometer showed two distinct deflections, the first indicating the time of arrival of the salt at the artery and the second the time of

arrival at the vein. This is a useful method of measuring the circulation time of an organ when the artery and vein run close to each other, as it avoids disturbance of the vessels by dissection.

2. *Injection into central end of left carotid.* Time to a tributary of the splenic vein near the spleen, 15.0, 16.3, 15.9 (average 15.7 seconds). Time from carotid to splenic artery, 3.5, 3.0 (average 3.3 seconds). Circulation time of spleen, 12.4 seconds.

3. *Injection through a cannula inserted into a branch of the splenic artery* toward the spleen. Time to splenic vein, 10, 10.1, 10 seconds (average 10 seconds). Later, after removing cannula and inserting a better one into the branch of splenic artery, time to splenic vein 11.7, 10.0, 9.1, 12.3, 12.2, 10.2, 13.0 seconds. Average circulation time of the spleen, 11.2 seconds. With injection so near the spleen, although a small quantity of salt solution was used for each injection, an excess of pressure might sometimes cause some shortening of the circulation time. Yet the agreement with the previous result with injection into the carotid is good for an organ like the spleen, whose circulation time is easily lengthened by cooling and exposure (vasoconstriction). It had been exposed and felt cold. Now warmed the spleen with sponges, and repeated the injections into the branch of splenic artery. The following times were obtained: 10.2, 10.6, 9.3, 10.3, 10.0, 12.4 (average 10.5 seconds).

Time from jugular to carotid again measured. At this period of the experiment strong general convulsions (cortical?) developed at intervals. The following times were obtained: 6.4 seconds, 4.6 seconds (during strong convulsions). After the fit was over, 6.4, 6.7, 7.0 seconds (a little over-estimated). During another attack salt solution was injected into the jugular at the height of the spasm and arrived so slowly at the carotid that no deflection was observed. Just at the end of the spasm the time was again 6 seconds; just after another fit, 4.5 seconds, and then in two observations without spasms, 6.1 and 6.8 seconds.

4. *Injection into the splenic artery* toward the aorta. Time to the left spermatic artery (isolated on the electrodes) 3.4 and 3.4 seconds in two observations. Time to left renal vein, 11.0 seconds and later on 17.4, 15.3, 14.2, 15.0 and 11.4 seconds. The kidney had been exposed and had cooled. These long and variable circulation times are associated with considerable vasoconstriction.

Orifice of cannula in carotid, 11 cm. from heart. Orifice of cannula in splenic artery, 9 cm. from the abdominal aorta. Distance from origin of splenic artery to the electrodes on the spermatic artery, 25 cm.; from the electrodes to the testicle, 5 cm. The average linear velocity of the blood from the splenic artery to spermatic artery in the above observations would be about 80 mm. per second, the circulation time of the testicle about 7 seconds. Distance from origin of splenic artery to origin of left renal artery, 5.5 cm. Average circulation time of kidney, about 13 seconds. The splenic artery came off from the abdominal aorta more than 1 cm. above the origin of the superior mesenteric.

To save the introduction of a large number of protocols, the values for the time of the lesser circulation in 23 dogs are given in table 3. The figures in each case are the average of a number of observations. As regards the times from the jugular or right heart to the artery, these

are not subject to any uncertainty apart from ordinary errors of observation and the naturally occurring variations in successive observations. In the dogs distinguished by roman numerals the minute volume was determined, and it is possible to calculate the quantity of blood in the lungs. It may be pointed out that the numbers for the gross circulation time are about equally accurate, for the given experimental conditions, in the different animals, being the averages of a fair number of actually observed times, which did not differ widely in the same animal. On the other hand, the numbers given for the net circulation time from the right heart to the aorta are of unequal value. Some were obtained by two measurements of the time, first, from the point of injection into the vein to the artery and then from the left ventricle to the artery. The difference of these two times, with sometimes a small allowance for time lost in the passage of the salt from the cannula or catheter to the right ventricle, is the correct net time, and these are the most accurate observations (e.g., dogs XX, XXII and XXIII). In others the net time has been obtained by using the time from the left ventricle to artery directly measured on other dogs of approximately the same size, in which the minute volume was the same and the distance of the electrodes from the heart (measured along the arteries) either the same or nearly the same. Where the gross time is corrected entirely by calculation of the probable time from left ventricle to artery from the length of the arterial path, the results of the observations with electrodes on the carotid may be presumed to be more nearly correct than where the electrodes are on the femoral, since the arterial path to the carotid is so much shorter. The smaller the animal, the smaller in general is the error in allowing for this by calculation. An increase in the net time of the lesser circulation with the weight of the animal is most obvious when the smallest dogs are compared with the largest. But for a considerable intermediate range no very definite relation to body weight is apparent. This may be due partly to the unavoidable differences in the experimental conditions, and partly to errors in calculating the net time.

In the animals in which determinations of the minute volume were available the quantity of blood in the lungs and the percentage of the total blood in the body (assuming it to be $\frac{1}{13}$ of the body-weight) were calculated. No great weight is to be attached to the absolute values obtained, and it is quite likely that some of the higher percentages may be somewhat, perhaps considerably, over-estimated. This would be the case if either the minute volume or the net time of the lesser

circulation were taken at too high a value. In general the percentages of the total blood constituted by the blood in the lungs are higher than have usually been assumed, and than the results of determinations on the ligated lungs of dogs by Plumier (7). This agrees with the suggestion of Kuno (3), based on his observations on the quantity of blood in the lungs when the circulation through the heart-lung preparation is varied, that the older estimates are too low. He got values varying from 8.8 to 19.4 per cent of the total blood, and in edema of the lungs as much as 26 per cent. Of course his conditions were artificial, but the general agreement with my results is suggestive. In 5 experiments on the heart-lung preparation of dogs he calculated the mean circulation times of the lungs as 3.8, 4.4, 1.8, 2.2 and 2.2 seconds, the body-weights being 7, 8.1, 4.6, 4.2 and 5.1 kgm. I have corrected his calculation for the second dog, the time given by him being 1.98 seconds. It should be 4.4. These figures cannot be compared with mine because the velocity of the circulation was artificially varied within a wide range by changing the venous pressure, and therefore the amount of blood entering the right heart. Also, the net times in table 3 are from right heart to aorta, while Kuno's calculated times are from pulmonary artery to pulmonary veins.

It is clear from table 3 that the circulation time, as determined by the injection method, cannot be much too short, since this would tend to cause the calculated percentage of blood in the lungs to be low, not too high. It would be plainly impossible to double, or to greatly increase these circulation times without obtaining absolutely preposterous percentages. Nor would any possible deduction for over-estimation of the minute volume enable us to materially increase the circulation times. It has been previously pointed out (23) that, with the procedure employed in estimating the minute volume in the great majority of the observations, errors due to failure to collect the blood specimen exactly at the right time would generally tend to bring out too large a calculated minute volume. It is possible therefore that in some of the observations this quantity may have been somewhat over-estimated, although apart from certain observations in which it was specifically noted that such was the case, there was no evidence of this. But if in any experiment such an over-estimation did occur, any allowance made for this ought almost certainly to be applied not to increasing the net pulmonary circulation time, but to diminishing the calculated quantity of blood in the lungs. Round numbers have been used purposely for the minute volume and the quantity of blood in the

lungs, the latter being arrived at after deducting the output of a ventricle per beat from the amount reckoned on the net time from right

TABLE 3

DOG	WEIGHT	LENGTH	DISTANCE OF HEART TO ARTERY	CIRCULATION TIME		MINUTE VOLUME PER KILOGRAM	CALCULATED	
				To artery	Right heart to aorta		Blood in lungs	Per cent. of total blood
	kgm.	*cm.*	*mm.*	*seconds*	*seconds*	*cc.*	*cc.*	
A	21.1			9.6	7.5			
XVII	18.2		130	10.4	8	140	280	20
B	13.3			7.8	6			
XVI	11.8		75	8.0	6	200	200	20
				10.2	7.5	95	130	15
C	9.8			9.2	7.5			
D	4.5			6.1	4.5			
E	3.2			6.4	4			
F	1.0			2.0	1.7			
XIV	32.2		550	14.0	8	150	580	24
				11.2	6	150	430	17
XIII	27.9			14.7	7	110	320	15
XXII	17.5	106	495	12.0	6.0	190	280	21
						150	200	15
XII	15.2	93	470	17.2	7	190	290	26
VI	13.3	88	420	15.3	7			
XIX	12.8	78	370	7.4	4	150	110	11
X	12.3		370	14.1	6	200	200	22
XI	11.7			12.8	5	200	150	17
XX	10.3	84	470	15.1	7.0	160	165	21
							130	17
XVIII	9.9	80	400	13.2	6	140	110	15
VIII	8.4		300	11.5	5	200	110	17
G	7.4		310	10.5	6			
XXIII	7.2	73	330	11.5	5.0	200	80	15
				15.0	5.0	150	65	12
VII	6.5		300	9.6	4.5	150	55	11
				6.4	3	280	60	12
XV	4.9		350	11.1	4	300	60	15

In dogs A to F inclusive and in dogs XVI and XVII the electrodes were on the carotid, in the others on the femoral artery.

ventricle to aorta. I desire to point out distinctly that the amount of blood in the lungs in this table is only calculated with the object of testing the question whether the time of the lesser circulation, as

determined by injection methods, is approximately the true average time or greatly less than the average time. These calculated values must always yield to values directly estimated by unexceptionable methods, if such exist.

Sometimes, as in dogs XVI and VII, the minute volume early in the experiment differed much from that obtained toward the end, either because of deterioration of the animal or because experimental conditions had been purposely or accidentally induced (*e.g.*, hydremic plethora) which were calculated to affect the output. It then depends upon the concomitant change in the pulmonary circulation time how much and in which direction the calculated quantity of blood in the lungs will vary. Thus, in dog VII the reduction in the time almost exactly compensated for the increase in the minute volume, and the calculated quantity of blood in the lungs remained practically unchanged.

Some direct estimations of the quantity of blood in the lungs. I made some experiments on dogs to determine the extreme range of the amounts of blood contained in the lungs under different conditions. The pulmonary artery (or right heart) alone, or the aorta (or left ventricle) alone was blocked by the sudden injection of melted paraffin through a catheter passed into the jugular vein or carotid artery. In some experiments an attempt was made to block both pulmonary artery (or right heart) and aorta or left ventricle simultaneously. The animals were of course completely anesthetized. The temperature of the paraffin was such that it would pass through the catheter readily and yet solidify rapidly on reaching the blood (57° to 60°C.). The precise distribution of the paraffin was determined *post mortem*. It proved easier to block the pulmonary artery than the aorta. A number of the experiments failed for various reasons, but a fair number were successful. The lungs were ligated and generally the heart also, and the blood in them estimated colorimetrically.

Experiment 1. Dog 9.97 kgm. The aorta was completely blocked, the right heart and pulmonary artery free. The lungs appeared much congested. The blood in the lungs was 169 gm. (22 per cent of the total blood).

Experiment 2. Bitch, 6.69 kgm. (free from stomach contents). Right heart completely blocked, also origin of aorta up to the semilunar valves. The coronary arteries were blocked to their terminations. The liver was much congested. Blood in lungs 109 gm. (21 per cent of total blood).

Experiment 3. Dog, 4.59 kgm. Pulmonary artery completely blocked, aorta partially blocked but not so as to completely prevent the passage of blood. The lungs were very pale. The heart continued to beat, as observed through the chest, for several minutes after the injection. The superior and inferior venae cavae

and the hepatic vein were blocked, but the right ventricle still contained 6 gm. of blood. The coronary arteries were blocked at their origin, but not their branches. The liver was much congested. The lungs contained 22.2 grams of blood (about 6 per cent of the total blood). The left ventricle contained 4 grams of blood.

Experiment 4. Dog, 6.23 kgm. (free from gastro-intestinal contents). Pulmonary artery completely blocked. Superior and inferior cavae blocked near the heart. Aorta almost, but not quite completely blocked. Blood in lungs, 42 grams (9 per cent of total blood). Blood in right ventricle, 8.5 grams; in left heart, 17 grams; in heart and lungs, 67 grams (14 per cent of total blood).

Experiment 5. Bitch, 8.16 kgm. Morphine and ether. Aorta blocked. Superior cava blocked, but inferior cava and its tributaries were free. The heart beat at the rate of 48 a minute for 3 minutes after the injection and for 10 minutes more at a rate increasing to 78 per minute, the beat becoming somewhat irregular. The chest was then opened. Blood in heart and lungs, 182 grams (30 per cent of total blood).

Experiment 6. Dog (young puppy), 0.93 kgm. (free from gastro-intestinal contents). Right auricle and ventricle blocked, but contain some blood. Superior cava blocked. Pulmonary artery and aorta completely blocked, also coronary arteries. Some paraffin in the left ventricle. The animal died immediately, with hardly a single respiration after the injection. Blood in lungs, 9 grams (18.6 per cent of total blood), in heart cavities (chiefly right side) 4.5 grams. Total blood in body (estimated by Welcker's method) 48.5 grams (or $\frac{1}{15}$ of body-weight).

Experiment 7. Dog, 9.01 kgm. (free from gastro-intestinal contents). Pulmonary artery and right ventricle blocked. Some paraffin in the right ventricle. Aorta fairly filled but not quite blocked. No paraffin in left heart. The unligated carotid was blocked. Blood in lungs, 49.5 grams (7 per cent of total blood). Blood in right heart, 20.2 grams; in left heart, 5.7 grams. The left heart was firmly contracted. Blood in heart and lungs, 75.4 grams (about 11 per cent of total blood).

Experiment 8. Dog, 8.0 kgm. (free from gastro-intestinal contents). Left ventricle blocked. No paraffin elsewhere. Blood in lungs and right heart, 165 grams (27 per cent of total blood).

Experiment 9. Dog, 7.2 kgm. (free from gastro-intestinal contents). Only right heart blocked. Blood in lungs, 20 grams (3.5 per cent of total blood).

Experiment 10. Dog, 11.2 kgm. (free from gastro-intestinal contents). Right heart blocked. Blood in lungs, 44 grams (5 per cent of total blood).

Experiment 11. In one experiment of this series on a young dog the respiration and heart beat continued for an extraordinarily long time after injection of melted paraffin, at about 60°C., into the right jugular and left carotid. The animal was anesthetized with morphine and ether. Injection was made into the vein a moment later than into the artery. The respiration was not immediately affected, at least not markedly. The beat of the heart could be felt through the chest wall for 57 minutes from the end of the injection. Respiration continued 52 minutes and was fairly regular, though rapid, until a few minutes before it stopped. Corneal reflex present till about 8 minutes before stoppage of the heart, and was well marked. Ammonia placed in front of the nose, about 47 minutes after the

injection, caused an increase in the rate of respiration, and the head was turned sharply away. Tapping the left leg caused prompt drawing up of the leg. There was no similar reaction in the right leg till a short time before stoppage of the heart, when it appeared here also. The iris did not react to light at any time after the injection. The tongue and gums were pale. There were no voluntary movements of the limbs, but slight movements of the head, and considerable movement of the tongue before each respiration. Necropsy was made immediately after the stoppage of the heart. The right auricle and superior cava up to the jugular were completely filled with paraffin. Much paraffin in the right ventricle, but it was not entirely filled. Pulmonary artery and all its branches completely blocked, the plugs extending some way into the lobes of the lungs, except in the case of the lowest lobe of the left lung, where the plug stopped just at the point of entrance of the branch of the artery into the lobe. The lungs contained a fair quantity of blood. The inferior vena cava was completely filled from the auricle down to within a short distance of the diaphragm. Below the diaphragm there was no paraffin in the inferior cava, except a plug about 40 mm. long, which could be moved, but completely filled the vessel at the level of the right renal vein. No paraffin in any of the tributaries of the inferior cava. The veins of the mesentery were intensely engorged, the arteries shrunken in comparison. The aorta was completely filled with paraffin from its origin down to and including the external iliacs, with the exception of a portion of the thoracic aorta extending from 5 cm. above the diaphragm to within 5 cm. of the top of the arch. This portion was entirely free from paraffin. No paraffin in the femoral arteries. Both subclavian arteries completely filled. Right carotid entirely filled up to the circle of Willis and a plug in the right posterior communicating artery. Internal mammary arteries both completely filled. Everywhere the paraffin was well solidified. I make no comment on this experiment. There can be no question about the facts. How could any circulation be maintained? That the paraffin may have remained unsolidified for a certain time is possible, and is indicated by the fact that for some time a pulsation was observed over the left femoral artery in the thigh.

In three experiments the blood in the lungs was estimated *post mortem* without injection of paraffin: in a dog bled to death, in a dog killed by passing the street current through the chest, and in a dog which died after double vagotomy in the neck.

Experiment 12. Dog, 9.2 kgm. Killed by bleeding from the carotid. Weight of lungs, 98 grams. Blood in the lungs, 21.6 grams (about 3 per cent of the total blood originally in the body). Weight of the blood-free lungs, 76.4 grams (8.3 gm. per kgm. of body-weight).

Experiment 13. Dog, 12.7 kgm.; 0.18 gram morphine. Killed by passing street current (110 volts) through the cardiac region. Sponge electrodes on shaved skin, one over the impulse, the other over the ribs on the right side. The current was passed continuously for 30 seconds, then made and broken rapidly 5 or 6 times. No pulse felt afterwards: two or three gasps. Chest opened. Heart enormously distended. Lungs fairly red. Heart muscle engorged with dark blood. The right auricular appendix continued to beat. The lungs were ligated off. Blood in lungs, 86 grams (9 per cent of the total blood). Blood in right heart, 75 grams, in left heart, 44 grams. Blood in coronary circulation,

about 13 grams. Weight of blood-free heart, 112 grams (8.8 gm. per kgm. of body-weight). Blood in heart and lungs, about 25 per cent of total blood.

Experiment 14. Dog, 3.52 kgm. Both vagi divided; death after 2 days. Blood in lungs, 33 grams (12 per cent of total blood). Blood in heart, 26 grams. Blood in lungs and heart, 59 grams (22 per cent of total blood.)

The object of the experiments was to determine the greatest and smallest quantities of blood contained in the lungs, or in the heart and lungs together, under conditions inducing pulmonary engorgement or ischemia. While this object may be considered as having been attained, the number of experiments in which simultaneous blocking of the inflow and outflow of the lungs could be assumed to have been successfully accomplished is far too small to permit conclusions as to the limits within which the quantity of blood in the lesser circulation may vary in ordinary physiological conditions.

Nor can it be claimed that the method was proved to be adequate for obtaining such data. Absolutely simultaneous blocking of right and left heart (or of pulmonary artery and pulmonary veins) is essential for determining the quantity of blood actually contained in the lungs at a given moment. But the pressure of the injection must be much higher for the catheter or cannula in the left ventricle than for that in the right ventricle. Two separate pressure bottles were employed, but the connecting tubes were opened simultaneously by a single movement of a hinged piece of wood which compressed them. The one advantage of the method is that the chest remains intact. Apart from the disadvantage of opening the chest, the method of tying off the lungs, as practiced by Plumier, has everything in its favor.

SUMMARY

If V is the minute volume of the heart in cubic centimeters, T, the mean circulation time of the lungs or of the lesser circulation in seconds, and Q, the average quantity of blood (in cubic centimeters) in the lungs or in the lesser circulation at the time when V and T are determined, then $V = Q \frac{60}{T}$. The approximate values calculated for Q in a series of dogs in which V and T were estimated are given. The values of Q are usually higher than those obtained by previous workers by direct estimation of the blood in the ligated lungs. Even if some deduction is made from V for possible over-estimation of that quantity, Q still comes out so high that it is not possible to assume, as Tigerstedt has done, that methods depending on injection of salts or pigments into the circulation give much too low a value for T, owing to the "hastening on" of a portion of the injected substance in the axial stream. In a

network of capillaries filled with blood corpuscles, it is not conceivable that the same particle of injected material should continue moving with the maximum velocity for more than a small fraction of the total circulation time, its path being necessarily an "out and in" one. If we were to increase T materially above the actually observed (corrected) time, Q would come out impossibly high.

Some experiments were made to estimate directly the extreme range of the amounts of blood contained in the lungs, or in the lungs and heart under different conditions.

When the outflow through the aorta was completely blocked, the inflow into the right heart being unobstructed or at least the inferior cava open, the lungs contained 22 per cent of the total blood in one animal, and the heart and lungs together 27 and 30 per cent in 2 animals. When the block on the right side was complete, or at least the pulmonary artery was entirely blocked, while the outflow from the left side of the heart was either entirely free or only partially obstructed, the lungs contained 6, 9, 7, 3.5 and 5 per cent of the total blood in 5 animals. When both sides of the heart were completely obstructed simultaneously, the lungs contained 21 per cent and 18.6 per cent in 2 animals. In an animal bled to death the lungs contained 3 per cent of the total blood. In an animal killed by passing a strong current through the heart the lungs contained 9 per cent, and the lungs and heart together 25 per cent of the total blood.

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POSSIBLE RELATIONS OF THE WEIGHT OF THE LUNGS AND OTHER ORGANS TO BODY-WEIGHT AND SURFACE AREA (IN DOGS)

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As stated in the preceding paper (1), the observations were made primarily with the idea that the weight of the lungs, particularly of the blood-free lungs, is probably roughly proportional to their average vascular capacity, since the thin pulmonary membrane is largely covered with capillaries. It was accordingly supposed that the relations of the lung weight to body-weight and surface area in a series of dogs varying greatly in size might indicate how the average quantity of blood in the lungs varies with size. It was planned at that time to make also direct determinations of the quantity of blood in the lungs in dogs of different size, data of this kind being, as far as I am aware, very scanty. For the reasons mentioned in the preceding paper, this program was not completed, but the data obtained on the weights of the lungs (and other organs) seem worthy of being recorded.

In twelve dogs varying from 36.7 kgm. to 2.7 kgm. the area of the skin was measured approximately. The animals were etherized and bled to death from the carotids. The skin was then removed as completely as possible, spread flat on a table, subcutaneous surface next to the table. It was not stretched but simply smoothly laid on the table. It was trimmed with a sharp knife so as to leave a large rectangle, and the portions trimmed off were pieced together, being cut when necessary, to form a number of smaller rectangles. The combined area was then measured. Everything except the measurement being done by an assistant, very little labor was required to get an estimate of the area, which was considered close enough for the purpose. The weights of the animals, free from gastro-intestinal contents, were also obtained. The bleeding was exhaustive, but no anticoagulant was employed and there was no washing out with salt solution. The organs were weighed after being simply dried with a towel or with blotting paper. The heart was opened and the interior wiped clean, and the same for the stomach and intestines. The bronchi were cut off the lungs as far into the lobes as was practicable without removing parenchyma. The great vessels were cut close to the heart, but subpericardial fat was not removed. The oesophagus and rectum were not included in weighing the stomach and intestines.

The results are given in tables 1 and 2. The organ weights of three dogs bled to death whose skin area was not determined are included in tables 3 and 4. It will be seen that, on the whole, the lung weights are more nearly proportional to skin area than to body-weight. The number of grams of lung per kilogram of body-weight increases in passing from the larger to the smaller animals. The same is true for the liver, the kidneys and the stomach and intestines. The spleen weight is much more nearly proportional to the body-weight than to the surface, the number of grams of spleen per 1000 sq. cm. of skin decreasing markedly in passing from the larger to the smaller dogs. The results are not quite so clear for the heart, but the relation to body-weight seems to be closer than to surface. This is more marked if the heart-weight of dog 10 (goiter heart) is omitted, as it should be. No doubt it would be better to estimate the residual blood in the various organs and thus obtain the weight of the completely blood-free organs. For our purpose, however, it was not thought worth while to incur the considerable labor necessary to do this, as it was not thought possible that any well-marked proportionality of organ-weight to surface or to body-weight respectively could be obscured by the inclusion of the small amount of residual blood. It must be noted that in addition to the large proportion of the total blood which in these animals was drawn off from the carotid, some blood escapes from the organs when they are excised immediately after death, and some of the residual blood is not in the organs but in the great veins. A more important drawback is the small number of animals, which does not permit of averages being obtained from groups of animals of about the same body-weight. The number of grams of blood drawn off per 1000 sq. cm. of skin and per kilogram of body-weight is given in the last two columns of table 2. There seems to be a somewhat closer relation to body-weight than to surface. But little importance can be attached to these values since no definite proportion of the total blood escapes. Some liquid may have entered the blood vessels during the hemorrhage, and this may account for the large proportion of the total blood drawn off. The rapidity of clotting is important. In another series of 7 dogs the ratios of body-weight to blood obtained were 24.7, 21.7, 20.7, 20.6, 18.6, 16.0 and 13.5. In the last dog clotting was very slow.

For the 8 dogs in tables 1 and 2 below 8 kgm. body-weight, the average weight is 4.86 kgm; the average number of grams of organ per kilogram is 8.8 for the lungs, 8.1 for the heart, (excluding one dog in which it is specifically noted that there was a large "goiter heart;" the weights

TABLE 1

DOG	BODY-WEIGHT	SKIN AREA	LUNGS	HEART	LIVER	SPLEEN	KIDNEYS	STOMACH AND INTESTINES	BLOOD RUN OUT	BODY-WEIGHT PER GRAM BLOOD	BODY-WEIGHT PER SQUARE CENTIMETER SKIN
	grams	*sq. cm.*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
1	36780	11600	225	245	680	120	132	1355	2435	15.1	3.17
2	26820	9640	171	213	472	69	89	1100	1450	18.5	2.78
3	18380	6590	136	111	618	37.5	76	748	1140	16.1	2.79
4	11300	5167	86.0	74.3	331	25.6	44.5	545	682	16.6	2.18
5	7705	3770	55.0	67.6	220	15.2	34.0	320	476	16.2	2.04
6	5755	3218	46.1	41.9	181	12.5	40.2	340	379	15.2	1.79
7	5400	2856	38.7	50.2	180	10.9	31.1	320	385	14.0	1.89
8	5250	3080	44.6	45.4	197	13.7					1.70
9	4615	2353	38.5	30.0	257	10.1	32.5	377	241	19.1	1.96
10	4055	2417	40.1	49.3	176	12.1	27.7	270	325	12.5	1.67
11	3340	1900	31.5	24.1	181	7.1	30.0	305	203	16.4	1.75
12	2755	2302	38.7	25.2	136	5.6	24.1	275	218	12.6	1.19

Dog 8 was a rather emaciated female with large thyroids. Bleeding not quite complete. Dog 9 was a male puppy. Dog 10 had large thyroids and large "goiter" heart. Dog 12 was a somewhat emaciated puppy with distemper and large thyroids.

TABLE 2

DOG	LUNGS PER		HEART PER		LIVER PER		SPLEEN PER		KIDNEYS PER		STOMACH AND INTESTINES PER		BLOOD DRAWN PER	
	1000 cm.² skin	Kilogram body-weight	1000 cm.² skin	Kilogram body-weight	1000 cm.² skin	Kilogram body-weight	1000 cm.² skin	Kilogram body-weight	1000 cm.² skin	Kilogram body-weight	1000 cm.² skin	Kilogram body-weight	1000 cm.² skin	Kilogram body-weight
	gms.	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*
1	19.4	6.1	21.1	6.7	58.6	18.5	10.3	3.3	11.4	3.6	117	36.8	210	66
2	17.7	6.4	22.1	7.9	49.0	17.6	7.2	2.6	9.2	3.3	114	41.0	150	54
3	20.6	7.4	16.8	6.0	93.8	33.6	5.7	2.0	11.5	4.1	114	40.7	173	62
4	16.6	7.6	14.4	6.6	64.0	29.3	4.9	2.3	8.6	3.9	106	48.2	132	60
5	14.6	7.1	17.9	8.8	58.3	28.6	4.0	2.0	9.0	4.4	85	41.5	126	62
6	14.3	8.0	13.0	7.3	56.1	31.4	3.9	2.2	12.5	7.0	106	59.1	118	66
7	13.6	7.2	17.6	9.3	63.0	33.3	3.8	2.0	10.9	5.8	112	59.2	135	71
8	14.5	8.5	14.7	8.6	63.8	37.4	4.4	2.6						
9	16.4	8.3	12.7	6.5	109.0	55.6	4.3	2.2	13.8	7.0	160	81.0	102	52
10	16.6	9.9	20.4	12.1	72.9	43.4	5.0	3.0	11.5	6.8	112	66.6	134	80
11	16.6	9.4	12.7	7.2	94.0	54.1	3.7	2.1	15.8	9.0	160	91.0	107	61
12	16.8	14.0	10.9	9.1	58.9	49.2	2.5	2.0	10.4	8.8	119	99.8	95	79

of the heart are liable to be less regular than those of the other organs in this goiter region); 39.5 for the liver, 2.2 for the spleen, 6.5 for the kidneys, 65.0 for the stomach and intestines. For the 4 dogs above 10 kgm. the corresponding numbers are: for the lungs 6.6, for the heart 6.9, for the liver 21.2, for the spleen 2.5, for the kidneys 3.4, for the stomach and intestines 40.2. These averages have been obtained by adding the organ-weights and dividing the sum by the number of kilos of combined body-weight. In this way the heavier animals in a group are of course "weighted" in comparison with the lighter ones. If instead of this, we take the sum of the number of grams of organ per kilogram of body-weight and divide it by the number of animals in a group, we get for the dogs below 8 kgm. the following averages: lungs 9.1, heart (excluding the goiter heart of dog 10) 8.1, liver 41.6, spleen 2.2, kidneys 7.0, stomach and intestines 71.1. For the 4 larger dogs the averages obtained in this way are: lungs 6.9, heart 6.8, liver 24.7, spleen 2.5, kidneys 3.7, stomach and intestines 41.7.

For the animals below 8 kgm. the average number of grams per 1000 sq. cm. of surface is: for the lungs 15.2, for the heart 14.2 (excluding the "goiter heart" of dog 10, or including this heart 15.0), for the liver 72.0, for the spleen 3.9, for the kidneys 12.0, for the stomach and intestines 122. For the dogs above 10 kgm. the corresponding averages are: for the lungs 18.6, for the heart 18.6, for the liver 66.3, for the spleen 7.0, for the kidneys 10.2, for the stomach and intestines 113. It is recognized that the number of animals above 10 kgm. is far too small, and had they been more numerous, at least two groups (say 10 to 20 kgm. and 20 to 40 kgm.) would be necessary.

In 12 additional dogs the weights of the lungs and heart, *minus* the blood contained in them (including the blood in the coronary vessels) were determined, the blood being estimated colorimetrically. The body-weights ranged from 4 to 16 kgm. For the group of 6 dogs between 4 and 8 kgm. inclusive, with an average body-weight of 6.5 kgm., the average number of grams of lung per kilogram of body-weight was 9.2; of heart 8.3. For the remaining 6 dogs, between 8 kgm. and 16 kgm. (average weight 11.2 kgm.), the corresponding numbers were for the lungs 7.1, and for the heart 8.0. The lungs, as weighed in this series, included somewhat more of the bronchi than in the series of tables 1 and 2.

In table 3 are given the organ weights in a number of dogs whose skin area was not determined, and in table 4 the number of grams of organ per kilogram of body-weight. I prefer not to attempt to calculate

TABLE 3

DOG	BODY-WEIGHT	LUNGS		HEART	LIVER	SPLEEN	KIDNEYS		STOMACH	INTESTINES	THYROID	
		Left	Right				Left	Right			Left	Right
	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams	grams
13	32260	89	115	246	623	77	70	64	340	775	7.5	8.0
14	27890	86	112	146	285	37	41	42	159	277	3.3	2.3
15	25270	88	113	266	794	76	58	61			10	16
16	18850			121	527	21	61	67	204	566	5.5	28.0
17	14775	54	64	112	600	39	57	54				
18	14043	141*		131	478	38	99*		161	402		
19	14040	57	107	152	489	43	104*		170	425		
20	13815			149	517	39	47	45	172	562	10	10
21	11325	45	65	110	453	20	37	39			1	2
22	9965	53	93	89	404	32	30	28			1.5	1.5
23	9524	86*		71	258	12.5	20.7	18.0			1.0	0.9
24	9060	127*		89	313	20	28	28			1.7	1.5
25	8830	102*			247	25	27	28			3	3
26	8616	125*		81	190	9.5	45*				7.5*	
27	8605	35	46	64	314	21	28	26			2.0	2.0
28	8155	92*		64	368	25	31	29			3	4
29	8154	90*		89	271	28	15.5	14.0	118	326		
30	7587	81*		68	278	15	60*					
31	7250			62	265	18	20	20			1.5	1.4
32	6480				329	16.8	52*					
33	5890	25	44	50	453	20	25	25			1	2
34	5436	29	39	77	156	12	20	19			2	2
35	5435	20	27	57	184	21	26.4	25.5			1.2	1.4
36	5175			60	484	11.5	40.7	40.5			2.7	2.5
37	4983	24	32	43	185	14	18.0	18.2				
38	4983	21	24	42	230	14	19	19	78	205	4*	
39	4416	22	30	38	198	16	18.3	19.2	453*		2.5	2.6
40	3850	14.5	18.5	34	125	7	11.2	10.5	55		2.2	2.5
41	3510	35*		35	152	5	24*		57	154		
42	3502	62*			121	7	26*					
43	3175	18	20	25.6	118	5.7	14.1	14.0			2.2	3.1
44	2378	43*		32	150		17	17				

Dog 14 was a very fat spayed bitch, bled to death. Dogs 16, 17, 20, 27, 39 and 40 had been subjected to considerable hemorrhage but were not completely bled. Dog 43 was bled to death.

* Where a number occupies this position both lungs, kidneys, etc., were weighed together.

the skin area from a formula involving body-weight only. But from the relation of organ weight to body-weight the general relation to surface area is sufficiently obvious. Most of the animals were not bled

TABLE 4

DOG	LUNGS PER KILOGRAM OF BODY-WEIGHT	HEART PER KILOGRAM OF BODY-WEIGHT	LIVER PER KILOGRAM OF BODY-WEIGHT	SPLEEN PER KILOGRAM OF BODY-WEIGHT	KIDNEYS PER KILOGRAM OF BODY-WEIGHT	STOMACH AND INTESTINES PER KILOGRAM BODY-WEIGHT
	grams	*grams*	*grams*	*grams*	*grams*	*grams*
13	6.3	7.6	19.3	2.4	4.2	34.6
14	7.1	5.2	10.2	1.3	3.0	15.6
15	7.9	10.5	31.4	3.0	4.7	
16		6.4	28.0	1.1	6.8	40.8
17	8.0	7.6	40.6	2.6	7.5	
18	10.0	9.3	34.0	2.7	7.1	40.1
19	11.7	10.8	34.8	3.0	7.4	42.4
20		10.8	37.4	2.8	6.7	53.1
21	9.7	9.7	40.0	1.8	6.7	
22	14.6	8.9	40.5	3.2	5.8	
23	9.0	7.4	27.1	1.3	4.0	
24	14.0	9.8	34.5	2.2	6 2	
25	11.5		28.0	2.8	6.2	
26	14.5	9.4	22.0	1.1	5.5	
27	9.4	7.4	36.5	2.4	6.3	
28	11.3	7.8	48.2	3.0	7.3	
29	11.0	10.9	33.2	3.4	3.6	54.4
30	10.7	9.0	36.6	2.0	7.9	
31		8.5	36.5	2.5	5.5	
32			50.4	2.6	8.0	
33	11.7	8.5	77.0	3.4	8.5	
34	12.5	14.1	28.7	2.2	7.2	
35	8.6	10.5	33.8	3.8	9.5	
36	11.6		93.5	2.2	17.6	
37	11.2	8.6	37.1	2.8	7.3	
38	9.0	8.4	46.2	2.8	7.6	76.8
39	11.7	8.6	44.8	3.6	8.5	102.5
40	8.6	8.9	32.5	1.8	5.6	
41	10.0	10.0	43.3	1.4	6.8	60.1
42	17.7		34.5	2.0	7.4	
43	11.9	8.0	37.1	1.8	8.8	
44	18.0	13.4	63.0		14.3	

to death, and this is reflected in the greater irregularity of the results than in tables 1 and 2. Yet it is seen that the same general relation of organ weight to body-weight exists. Thus, the average number of

grams of lung per kilogram of body-weight for all the dogs below 10 kgm. is 11.8, and for the dogs above 10 kgm. it is 8.6; for the heart the averages are 8.8 and 8.6; for the liver (excluding the exceptionally large liver of dog 36) 40.3 and 29.8; for the spleen 2.5 and 2.2; for the kidneys (excluding the exceptionally large kidneys of dog 36) 7.2 and 5.8. In calculating the proportional weights of the liver, spleen and kidneys the results on 3 dogs, in addition to those given in tables 3 and 4, are used. The weights of these animals were 21.08, 13.00, and 3.18 kgm. The livers weighed 26.8, 25.7 and 56.0 grams per kgm.; the spleens, 1.4, 2.0, and 2.9 grams per kgm.; the kidneys, 5.7, 4.4 and 7.9 grams per kgm. respectively.

In a cat weighing 3.39 kgm., bled to death, the lungs, prepared as described for the dogs in tables 1 and 2, had a weight of 6.6 grams per kgm. of body-weight. In 3 cats weighing 3.38, 2.98 and 2.45 kgm., the number of grams of liver per kilogram was 26.3, 49.3 and 31.0 respectively; of spleen 1.9, 4.0 and 1.4; of kidneys 6.0, 11.9 and 8.6. In 12 additional cats weighing 3.95, 3.32, 3.16, 3.06, 2.97, 2.78, 2.7, 2.53, 2.52, 2.5, 2.2 and 2.2 kgm., the corresponding numbers for the liver were 31.5, 26.2, 23.8, 30.4, 19.9, 22.9, 20.4, 30.9, 27.0, 24.0, 42.0 and 31.8 respectively.

In three rabbits weighing 1.77, 1.58, and 1.44 kgm. respectively, the numbers for the liver were 53.1, 35.6 and 36.4; for the spleen 0.5, 0.8 and 1.0; for the kidneys 7.3, 8.3 and 8.7. In 4 rabbits weighing 2.66, 1.80, 2.0 and 2.51 kgm., the liver weights were 22.5, 33.9, 41.0 and 15.0 grams per kgm. respectively. Cats and rabbits are not suitable for studying the relations between organ weight and body-weight or surface, if such exist, because of the small range in size.

Richet (2) weighed the liver and spleen of a large number of dogs without bleeding, and collected in addition observations of other writers. Apparently calculating the surface area from the weight, he arrived at the conclusion that the weight of the liver is proportional to the surface and that of the spleen to the body-weight.

Maurel (3), after Richet, studied the relation of the liver weight to body-weight and surface in different species of animals. Magnan (4) investigated the relation of the weight of the liver, kidneys, spleen, heart and lungs to the body-weight. According to him the weights of the liver and spleen are related to the nature of the food, and he denies any special significance in the relation of liver weight and surface. However he did not deal with the relations in individuals of the same species of very different size, where differences in alimentary habits could have no influence. And as Richet points out, it is a striking thing that when a large number of dogs of very different size are arranged in groups according to weight, the average weight of the spleen per kilogram of body-weight varies only from 2.42 to 2.90 grams for the different groups, while the average weight per 1000 sq. cm. of surface varies from 3.9 to 8.1 grams. For the liver the weight per kilogram varies from 22 to 42 grams in going from the largest to the smallest animals, and the weight per 1000 sq. cm. of surface only from 65 to 67 grams.

E. Voit (5) has pointed out how greatly the varying proportion of fat in the entire animal and in the individual organs can affect the ratios between organ weights and body-weight. Considerable irregularities in these ratios in a series of animals taken at random are therefore inevitable. In large series it is to be supposed that the influence of such irregularities will be in a great measure eliminated, when the averages are deduced from sufficiently large groups classified according to weight.

SUMMARY

In a series of dogs varying greatly in weight, the weight of the spleen was found to be proportional to the body-weight, that of the liver more nearly proportional to the surface. These observations on organs largely freed from blood confirm Richet's results on organs weighed with the blood in them. The weight of the stomach and intestines, freed from contents, is more nearly proportional to the surface than to the body-weight. The same is true of the kidneys. The weight of the completely blood-free lungs, or of the lungs largely freed from blood by exhaustive hemorrhage, appears to be more nearly proportional to the surface than to the body-weight. The heart weight seems to be more nearly proportional to the body-weight than to the surface. But in connection with the heart weights it should be noted that the material was derived from the goiter region of the Great Lakes, although obviously hypertrophied hearts were excluded.

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SYSTEMIC EFFECTS OF THE INTRAVENOUS INJECTION OF SOLUTIONS OF VARIOUS CONCENTRATIONS WITH ESPECIAL REFERENCE TO THE CEREBROSPINAL FLUID

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Two years ago Weed and McKibben (29) reported in this journal the effect of the intravenous injection of solutions of various concentrations upon the pressure of the cerebrospinal fluid. It was shown that such administration of strongly hypertonic solutions markedly lowered the pressure of the cerebrospinal fluid, frequently producing negative values; with hypotonic solutions (distilled water) a prolonged rise in the pressure of the fluid was obtained. Ringer's solution in large doses produced a temporary increase in the fluid pressure, followed quickly by a return to normal. Associated with these changes in the pressure of the cerebrospinal fluid were marked alterations in the volume of the brain (30), the hypertonic solutions producing a small shrunken brain while the hypotonic solutions caused an outspoken swelling of the brain substance. These experimental changes in brain bulk were particularly outspoken in animals in which the skull had been trephined and the dura opened.

Following the publication of these papers, there has appeared a number of other contributions to the subject, in which the general physiological findings have been confirmed and somewhat extended. Haden (13) in a short note in September, 1919, reported the suggestive observation of an amelioration of the increased intracranial tension in two meningitis patients following the intravenous injection of a 25 per cent solution of glucose. Cushing and Foley (5) in May, 1920, announced before the Society of Experimental Biology and Medicine the clinical application of the decrease in brain bulk following the administration of hypertonic solutions. In addition these observers demonstrated the significant fact that similar reductions of the pressure of the cerebrospinal fluid could be obtained after the ingestion of strongly hyper-

tonic solutions. With the intra-intestinal administration of distilled water a rise in the pressure of the fluid was obtained; this was not as striking nor as sustained as the changes in pressure due to the hypertonic solutions. Following the observations of Cushing and Foley, Sachs and Belcher (22) made a preliminary report of the use of a saturated solution of sodium chloride for the control of intracranial tension in a patient suffering from a cerebral tumor; success in decreasing the abnormal tension followed the intravenous injection of this hypertonic solution. This report preceded but shortly the detailed account of the experimental observations of Foley and Putnam (10), who confirmed the initial physiologic observations of Weed and McKibben and worked out in detail the changes in the pressure of the cerebrospinal fluid after intra-intestinal administration of solutions of various concentrations. Further clinical observations were made by Ebaugh and Stevenson (8) who had an opportunity to study intracranial tension in an epileptic with a subtemporal decompression. These authors reported on this patient a marked fall in intracranial tension following the intravenous and oral administration of hypertonic solutions and an increase in pressure after the oral administration of water (4000 to 8000 cc.). These results were controlled by giving Ringer's solution in similar quantities by mouth; a maximum alteration of only 5 mm. in the intracranial tension in the observation period was noted. The latest contribution to the subject was made by Sachs and Malone (23) who reported at the meeting of the American Physiological Society at Christmas, 1920, experimental observations on brain volume following the intravenous injection of 30 per cent solutions of sodium chloride. The decrease in brain volume under the conditions of observation was noted within 10 minutes with the maximum shrinkage occurring within 45 minutes to 1 hour. The effect of the hypertonic salt endured for hours; the exact persistence of the decreased volume was not determined. Sachs and Malone also summarized their findings in regard to arterial changes occurring in both animals and in patients, noting a significant difference dependent upon the rate of administration of the hypertonic solution.

In the first paper of Weed and McKibben (29), it was stated that observations upon the arterial and venous pressures in relation to the cerebrospinal fluid had been begun and would form the basis of a further report. These earlier experiments, but few in number, were carried out in the Army Neurosurgical Laboratory, the staff of which was assisted by Dr. D. R. Hooker; with the demobilization of this

laboratory force, the further continuance of the observations was necessarily postponed. But during the past fifteen months it has been possible to return to these interrupted studies and to carry out the original plans for their completion. The work of other observers has subsequently given data which have been needed for the elucidation of certain phases of the question but the larger problems underlying the phenomena have remained unattacked. It is the purpose of this paper, then, to record in detail the general systemic effects of the intravenous injection of solutions of various concentrations; the following paper will deal with the cranium as a rigid container in which exist blood, brain and cerebrospinal fluid; and the third will be devoted to the relation of the intracranial venous pressure to the pressure of the cerebrospinal fluid, particularly as affected by the intravenous injection of solutions of various concentrations.

METHODS OF INVESTIGATION

Cats were used entirely for this series of observations. No special preparations of the animals were made with the single exception that food was withheld throughout the day of the experiment. The anesthetized animals were placed lying on their sides upon a suitable board and the body-temperature was maintained by adjustment of an electric lamp.

Ether was the anesthetic chosen for all of the crucial experiments. In a limited number of cases, other non-volatile anesthethics (urethane, chloretone, luminal sodium) were employed but the impossibility of maintaining unvarying pressures of the cerebrospinal fluid throughout control observations with these narcotics led to their discontinuance. The earlier observations were made under ether administered by the method of intratracheal insufflation; by this means an unvarying pressure of the fluid could be maintained by proper regulation of the apparatus before the beginning of the experimental procedures. In the later experiments this method was superseded by the more simple procedure of introducing a tracheal cannula and connecting this cannula to a Woulfe bottle containing ether. Proper regulation of the proportions of air and ether in this bottle was made before the experimentation was started; it was held essential for the proper conduct of the experiment that the anesthetic should be uniform throughout. Whenever it was necessary to readjust the ether after the experiment had actually begun variations from the usual reactions were met with constantly.

The pressures of the cerebrospinal fluid were obtained by the introduction of a shortened lumbar puncture needle (usually 17 G) into the cisterna cerebellomedullaris through the occipito-atlantoid ligament and the connection of this needle to a manometer. As many of the experimental procedures gave negative pressures of the cerebrospinal fluid, a graduated U-manometer of 1 mm. bore was employed; this was customarily filled to the zero level with Ringer's solution. In some of the experiments the manometer was filled to practically the level of normal pressure; in others the fluid lost in connecting the needle with manometer was replaced. In a few observations no replacement of fluid was made but the readings were not taken until the period of compensatory rise was finished. All three of these methods gave practically the same initial levels of fluid; no experimental procedures were carried out until the pressure of the cerebrospinal fluid in the control period had remained constant for a period of from 5 to 15 minutes. The pressures of the cerebrospinal fluid were read directly from the manometer in terms of millimeters of fluid or Ringer's solution; the intervals between readings for this and all other pressures were customarily of 1 minute duration but occasionally readings every 30 seconds were made. We discarded all observations in which free pulsation of the cerebrospinal fluid in the manometer did not occur; the routine preparation afforded excursions of the fluid of at least 4 mm. for the respiratory change and of 1 mm. for the cardiac impulse. Likewise, if a blood-stained cerebrospinal fluid was obtained by the puncture, the experiment was discontinued.

The arterial pressure was recorded in the customary way by insertion of a cannula into the carotid artery and connection of the cannula with a manometer. In some of the earlier experiments a membrane manometer was used, being calibrated before and after the readings; later the simple mercury U-manometer was employed. For the greater proportion of observations, carotid pressure was recorded but in a few instances arterial readings were taken from the femoral. A continuous record of arterial pressure was taken throughout the initial period of control and throughout the period of injection of foreign solutions and thereafter for a number of minutes. Following this interval of abrupt arterial change, records were made every 5 minutes except when marked alterations were observed, or when for other reasons a continuous record was desired.

Systemic venous pressures were customarily recorded by the insertion of a cannula proximally into the superficial brachial vein and

connection of this cannula with a straight manometer of 1 mm. bore. The apparatus was arranged so that whenever desired a small amount of Ringer's solution could be run into the vein, the resultant level indicated in the manometer being read off directly in terms of Ringer's solution. Control observations showed that the amount of fluid thus injected was too small to affect the pressures recorded in the slighest degree. In a few experiments pressures were taken from the femoral and from the internal jugular veins.

The output of urine under the conditions of observation was determined by counting the drops flowing from a cannula introduced into the apex of the bladder after ligature of the urethra. This method gave data of qualitative but not of absolute value; it sufficed for the recording of periods of increased or decreased urinary fluid output.

All injections of foreign solutions were given from a burette connected to a cannula introduced into a vein—usually the superficial brachial. The rate of injection was adequately controlled by regulation of a Hoffman pinch-cock on the rubber tubing connecting burette to cannula.

The observations were carried out over periods of varying length, dependent upon the type of experiment. The longest period of experimentation was of 7 hours' duration, with readings made every minute. For the routine experiments a 2-hour interval was selected and the observations compiled in charts, with plotting of the pressures every minute. Other procedures employed will be noted in appropriate sections of this paper.

CONTROL OBSERVATIONS

A considerable number of observations was made on etherized animals in which the experimental procedures were limited to the operative measures necessary for the attachment of the various manometers for recording the arterial, venous and cerebrospinal fluid pressures and the urinary output. Under these conditions readings of the pressures were taken at minute intervals for periods up to 4 hours. Chart 1 gives the record of an experiment of this type and will serve as a control for the interpretation of the findings in other experiments to be discussed later.

In chart 1 it will be noticed that the pressure of the cerebrospinal fluid varied within very slight limits, the highest reading of the pressure (133 mm.) being but 11 mm. above the lowest (122 mm.). Throughout the 2 hour period of observation the cardiac and respiratory pul-

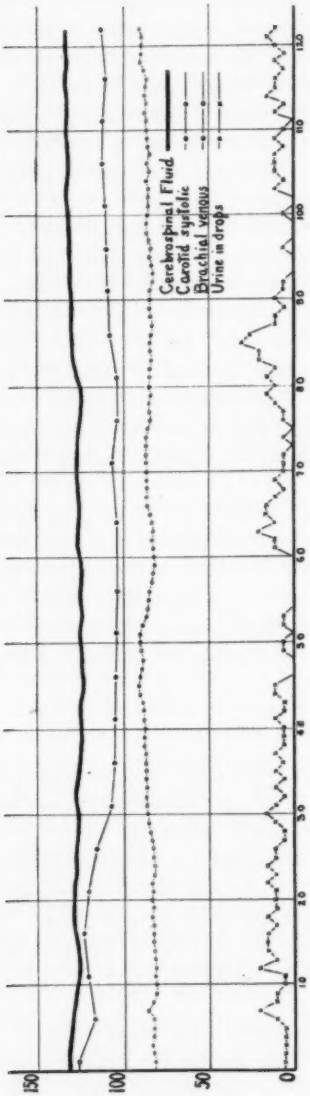

Chart 1. Experiment 61. Cat, weight 3200 grams. Ordinates represent millimeters of Ringer's solution or mercury (carotid pressure) and drops of urine (50 corresponding to 10 drops); abscissae represent time in minutes. Ether control.

sations of the fluid were wide (1 mm. and 4 mm.), indicating that free communication between manometer and subarachnoid space existed and conforming to the standard set to insure accuracy of the readings. The curve of fluid pressure is not unlike the record of normal pressure presented by Weed and McKibben (29) in their figure 2; it lacks, however, the initial rise to the sustained level. The absence of this initial rise in pressure in chart 1 is to be referred to the fact that the observations were not started until 5 minutes after introduction of the needle into the subarachnoid space. This record of the pressure of the cerebrospinal fluid demonstrates the standard of unvarying pressures which may be obtained under ether anesthesia; it establishes also a standard upon which deductions of experimental alteration in fluid pressures may be based.

The brachial venous pressure in the same experiment (chart 1) likewise showed but little variation throughout the period of experimentation. Starting at a pressure slightly above 80 mm. of Ringer's solution, the readings did not rise above 90 mm.; the lowest record for the 120 minutes was 81 mm. Slight variations in this venous pressure were not reflected in the cerebrospinal fluid; conversely the variations in the pressure of the fluid had no effect on the venous tension. The carotid systolic pressure taken at 5-minute intervals in this cat underwent more variation than did either the cerebrospinal fluid or the brachial venous. The changes in arterial tension however were slow and had no influence, if judged by the unvarying levels of the other two pressures, upon either. Urinary output as computed roughly by the number of drops per minute was somewhat irregular but continued uninterruptedly throughout.

Chart 1, the record of a typical experiment, affords then a fair standard of control for the subsequent experimental observations. Both the cerebrospinal fluid and the brachial venous pressures varied slightly but the variations were never abrupt. The avoidance of such abrupt changes in tension in these systems may be referred to the establishment of adequate experimental conditions, to the maintenance of the body heat of the animal and to the unvarying degree of anesthesia. Alteration of the concentration of the ether administered inevitably, as is well known, gives marked variation in the tension of the cerebrospinal fluid. The results of the present series of experiments confirm the view expressed by Weed and McKibben (29) that under proper experimental conditions the pressure of the cerebrospinal fluid does not vary greatly, the fluctuations being minimal in extent and rather slowly effected.

In the earlier experiments upon which this report is based use was made of the common non-volatile anesthetics. Our experience with urethane was quite typical of our findings with chloretone and sodium luminal. Three cats were given, by stomach tube, urethane in aqueous solution in identical dosage per kilogram of body-weight. In the first of these cats the pressure of the cerebrospinal fluid was initially recorded at 138 mm.; it then rather slowly but irregularly rose during the next 205 minutes reaching a maximum of 230 mm. in this time. From this point the pressure receded gradually, showing wide fluctuations of 5 to 35 mm., dropping finally to the reading of 180 mm. at the end of 6 hours. The second cat under apparently identical conditions gave an initial value of 123 mm. for the pressure of the cerebrospinal fluid; this pressure slowly but continuously decreased for 160 minutes and then recovered somewhat at the end of 6 hours. No wide fluctuations were recorded. In the third animal a marked and rapid increase in tension of the cerebrospinal fluid occurred, rising from the initial reading of 104 mm. to 183 mm. in 90 minutes at which time the animal suddenly died. The experience with these control animals under urethane anesthesia and similar results with the other non-volatile narcotics demonstrated that more reliable data, under constant experimental conditions, could be obtained by the proper regulation of ether as the anesthetic. Occasionally, however, we have had almost ideal experimental conditions in cats anesthetized with sodium luminal, but variations in degree of the anesthesia with similar doses prompted its discontinuance.

Normal pressures of cerebrospinal fluid. Numerous observers have reported average pressures of the cerebrospinal fluid obtained under various experimental conditions. In the earlier observations (those of Key and Retzius (17), Bergmann (2), (3), Falkenheim and Naunyn (9), Leyden (19)) considerable variation was reported but in the later work on the ordinary experimental mammals a fairly uniform level has been established. Dixon and Halliburton (7) gave 40 to 70 mm. of Ringer's solution as fair average of the fluid pressure in the dog under urethane-morphine anesthesia. Weed and McKibben (29) reported an initial pressure of 117 mm. of fluid for cats under ether by intratracheal insufflation; this value was increased to 129 mm. by an initial rise in pressure, accounted for by the replacement of fluid displaced into the manometer or lost in making the necessary connections. Becht (1) reported an average initial pressure of 112 mm. in animals under intratracheal ether, while Foley and Putnam (10) gave an average of

127 mm. for the normal reading in animals under ether. Their average value for initial readings on 100 cats under various anesthetics was 133 mm. of cerebrospinal fluid. Dixon and Halliburton and also Becht comment upon the wide fluctuations in pressure of the cerebrospinal fluid which occurred under their conditions of experimentation; such fluctuations seem to afford no indication of normal changes in the fluid pressure but are to be referred to factors in the experimentation.

In the present series of experiments on which this report is founded initial pressures from 77 cats under ether administered by the Woulfe bottle are available for the determination of the normal level. The average of these readings is 119 mm. of cerebrospinal fluid—a value coinciding rather closely with recent findings. As practically none of our experimental readings were begun until 5 minutes after puncture, the difference between initial reading and subsequent level, as noticed by Weed and McKibben (29), does not appear in our records.

While the average value of the pressure of the cerebrospinal fluid in these experiments was 119 mm. considerable variation from this mean was recorded. The maximum reading was 159 mm. and the minimum was 83 mm. of cerebrospinal fluid. In no case, in many hundred experimental punctures in animals, have initial negative values of the cerebrospinal fluid, such as reported by Foley and Putnam (10), been obtained.

RINGER'S SOLUTION

With the establishment of a definite standard of experimentation in which the animals were maintained in conditions of uniform pressures, observations upon the systemic effects of intravenous injections of relatively large amounts of Ringer's solution were made. It had been shown by Weed and McKibben (29) that the intravenous injections of large quantities of this isotonic solution caused a temporary increase in the pressure of the cerebrospinal fluid with a rapid return within 30 minutes to normal levels. With small doses of the Ringer's solution administered quite slowly, practically no effect on the cerebrospinal fluid was observed. In this present series of observations, the effects of intravenous injection of large doses of Ringer's solution were studied as the experiments constitute a control for comparison with the effects of injections of similar quantities of other solutions. The isotonic solution used was a modification of the original Ringer formula made up as follows:—NaCl, 0.9 per cent; $CaCl_2$, 0.025 per cent; KCl, 0.042 per cent; in distilled water.

Chart 2 gives graphic representation of the systemic effects of the intravenous injection of 50 cc. of Ringer's solution into a cat. In the initial control period of 11 minutes the cerebrospinal fluid showed a uniform pressure slightly below 150 mm; the brachial venous pressure was constant at between 72 and 74 mm. of Ringer's solution. During this interval the carotid systolic pressure ranged between 118 and 120 mm. Hg.; there was no urinary output. The injection of Ringer's solution was made at the rate of 10 cc. per minute for 5 minutes, and the recorded pressures all showed variations during this period. The cerebrospinal fluid rose rapidly in pressure from 147 to 195 mm., and the brachial venous pressure increased from 72 to 142 mm.—quantitatively a greater increase than that exhibited by the cerebrospinal fluid and a more pronounced reaction than was usually observed. During this period of injection the carotid systolic fell from 118 to 88 mm. Hg. On cessation of this very rapid introduction of fluid, the cerebrospinal fluid and the brachial venous pressures rapidly decreased, the former reaching a point but slightly above its control level in 17 minutes. In the same period the brachial venous pressure returned to its former level but then continued to fall slightly lower, maintaining a fairly constant pressure between 60 and 50 mm. throughout the remainder of the period of observation. The cerebrospinal fluid pressure remained slightly above the initial value (less than 10 mm.) for about a half-hour longer but then returned to exactly the preinjection level for the remainder of the experiment, the curve of pressure being practically level for 70 minutes. The fall in arterial pressure during the period of injection of the Ringer's solution was continued for 4 minutes afterwards, to be followed by a rather slow recovery to a point somewhat below its previous level. From this high point of recovery the carotid pressure fell gradually for the next 40 minutes, and then recovered slightly, maintaining a fairly constant level about 20 mm. below the initial pressures recorded.

Such a record as given in chart 2, which can be directly compared with figure 3 given by Weed and McKibben (29), permits the statement that the intravenous injection of a relatively large amount of a solution isotonic with the blood causes no prolonged disturbance of the pressure of the cerebrospinal fluid. The alterations in the carotid systolic and brachial venous, while marked during the period of injection, were not continued for any long interval; both of these pressures in the final adjustment were maintained at levels below those of the control period. The initial changes in pressure during the period of

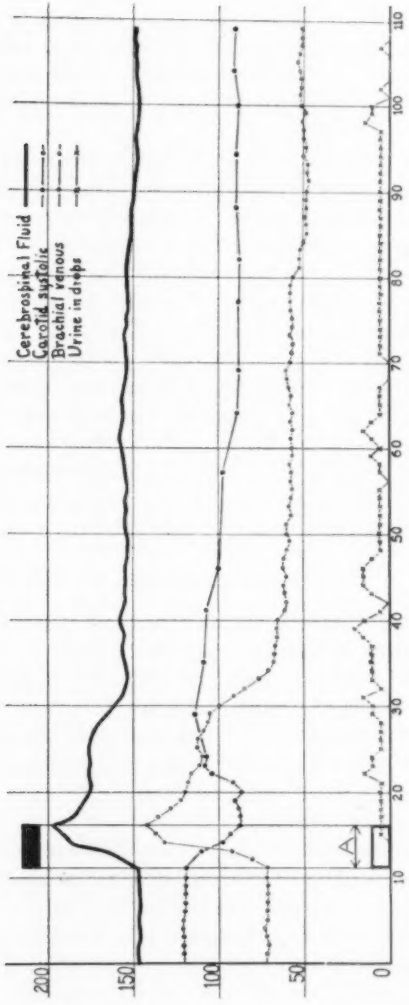

Chart 2. Experiment 44. Cat, weight 2750 grams. Ordinates represent millimeters of Ringer's solution or mercury (carotid pressure) and drops of urine (50 corresponding to 10 drops); abscissae represent time in minutes. During interval *A*, intravenous injection of 50 cc. of Ringer's solution.

introduction of the foreign solution were marked and were apparently to be referred, at least in part, to the impact and speed of the injection; the close correspondence of the cerebrospinal fluid curve and that of the brachial venous pressure is worthy of note. The urinary output in this animal was fairly constantly maintained after the cessation of the injection; it was not markedly different from the record of output given in chart 1.

The general physiological reactions obtained in the arterial and venous systems in these experiments are quite similar to the findings of many workers in this field. It has become quite well established that the arterial pressure cannot be maintained at a higher level by the injection of physiological saline; the compensation for the increased volume of fluid is brought about within a short interval (Dastre et Loye (6), Cohnstein und Zuntz (4), Johansson und Tigerstedt (16), Grosglik (11), R. Tigerstedt (28), C. Tigerstedt (27), Selig (24) etc.) though with relatively enormous doses the ultimate readjustment may be prolonged for a few hours. The general reactions noted here are by no means novel, but the general curve of the cerebrospinal fluid permits the use of this reaction as a basis for the interpretation of the effects brought about by the injection of solutions whose concentration of salts diverges widely from that of the blood.

HYPOTONIC SOLUTIONS

The marked increase in the pressure of the cerebrospinal fluid following the intravenous injection of a hypotonic solution (distilled water) noted by Weed and McKibben (29), has in the last 2 years been confirmed by other observers (Cushing and Foley (5), Foley and Putnam (10), Ebaugh and Stevenson (8)). The results of the present series of experiments are in accord with those reported 2 years ago by Weed and McKibben; the charts of the pressures of the cerebrospinal fluid coincide with their figures 4 and 5.

The record of the pressures obtained in a typical experiment in which the change of pressure of the cerebrospinal fluid was not extreme, is given in chart 3. Here during the preliminary control period of 8 minutes, the cerebrospinal fluid pressure was constant at 110 to 111 mm., while the brachial venous pressure hovered about 70 mm. The carotid systolic pressure was high—164 to 166 mm. Hg.; the urinary output, though somewhat greater than usual, was not abnormal. An intravenous injection of 50 cc. of distilled water was then given at the rate of 5 cc. per minute—a rate half that shown in chart 2. From the

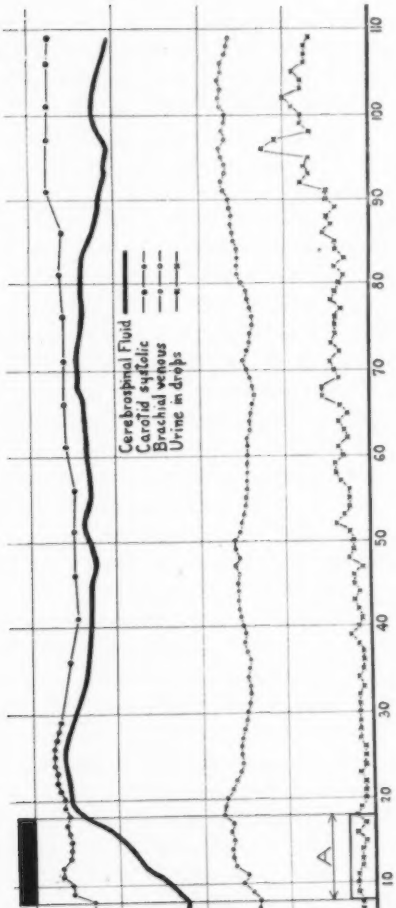

Chart 3. Experiment 51. Cat, weight 3250 grams. Ordinates represent millimeters of Ringer's solution or mercury (carotid pressure) and drops of urine (50 corresponding to 20 drops); abscissae represent time in minutes. During interval *A*, intravenous injection of 50 cc. of distilled water.

beginning of the injection the carotid, brachial venous and cerebrospinal fluid pressures all rose but at markedly different rates. The cerebrospinal fluid increased in pressure from 110 to 170 mm. during the introduction of the water; it continued to increase in pressure for some minutes thereafter, reaching a maximum of 182 mm. This heightened pressure of the fluid was maintained in part, the pressure showing slow waves of fluctuation; at the end of the 2 hour interval the pressure of the cerebrospinal fluid was still 45 mm. above the initial reading. The brachial venous pressure mounted during the period of injection but only from 68 to 89 mm.; it dropped back to its former level within 15 minutes; thereafter throughout the remainder of the observation the brachial venous pressure showed slow waves, at times carrying the pressure above its initial readings, and at times below. The carotid systolic pressure rose somewhat abruptly during the first part of the interval of injection and then fell, to rebound slightly. At the end of the injection this pressure was 179 mm. Hg. as compared with the initial reading of 165 mm.; this rise was followed by a further slow increase to 188 mm. after 8 minutes more. The carotid pressure then dropped back slowly to rise by gradual slight increments, making the whole curve of carotid readings show a slightly increased pressure following the injection. The urinary output showed but little tendency to increase during the first hour of observation but during the second hour a well-marked polyuria occurred.

While chart 3 may be considered as a typical reaction to the experimental injection of 50 cc. of distilled water in a cat, more extreme results have been frequently obtained, especially in young animals. Chart 4 is included here as giving such an outspoken reaction; in this animal the injection of 50 cc. of distilled water was made intravenously during a similar period of 10 minutes. The pressure of the cerebrospinal fluid mounted from approximately 125 mm. in the control period to 283 mm., this maximal reading being taken 12 minutes after the completion of the injection. The pressure then receded somewhat, to rise again, and finally to fall slowly; at the end of this period of observation (65 minutes) the cerebrospinal fluid pressure registered 232 mm.—107 mm. above the initial reading. The brachial venous pressure, in this animal, being initially taken at slightly above 100 mm., rose somewhat slowly during the period of injection and continued to increase for 7 minutes thereafter, reaching a maximum of 174 mm. Then it gradually decreased to attain a new level at slightly above 110 mm., which it held until the end. The slight increase in

carotid systolic pressure, recorded during and after the interval of injection, is of the same magnitude as that of chart 3.

In both chart 3 and chart 4 there is exhibited a distinct tendency for the pressure of the cerebrospinal fluid to return slowly to the initial or normal pressure shown before injection of the hypotonic solution. In neither animal, however, was a complete recovery achieved; in other cases the 2-hour period included a phase of complete return to the

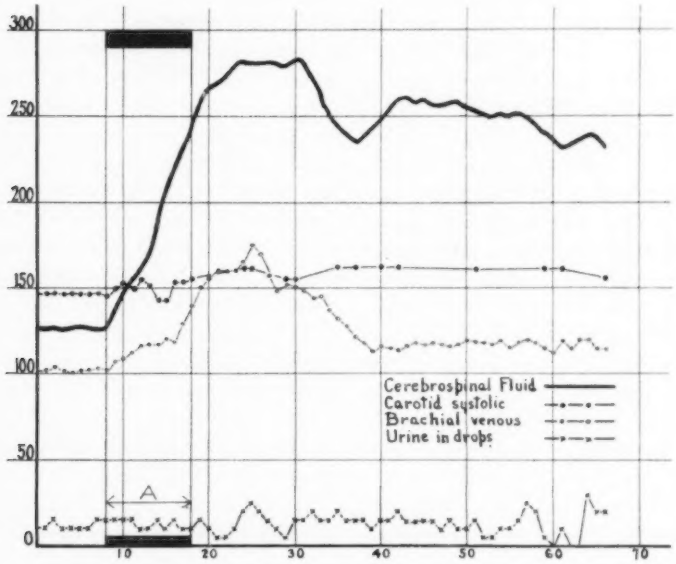

Chart 4. Experiment 40. Cat, weight 1700 grams. Ordinates represent millimeters of Ringer's solution or mercury (carotid pressure) and drops of urine (50 corresponding to 10 drops); abscissae represent time in minutes. During interval *A*, intravenous injection of 50 cc. of distilled water.

normal tension. In still other animals the increase in the pressure of the cerebrospinal fluid was followed by a drop to levels below the initial readings, at times as far as 20 mm. below the normal; such reactions were however rare and have occurred in only a small number of the cats in this series. With such great individual variation in recovery time, a number of cats was used to investigate the question, the observations being continued over periods up to 7 hours. Such an experiment is

given in chart 5, in which are given only the pressure of the cerebrospinal fluid and the output of urine in drops. The observations are here plotted at 5-minute intervals over a period of 6½ hours; the chart therefore cannot be directly compared with others in this paper. The initial pressure of the cerebrospinal fluid of slightly over 120 mm. was increased by the injection to 300 mm., the highest reading being obtained 100 minutes after the injection was completed. From this

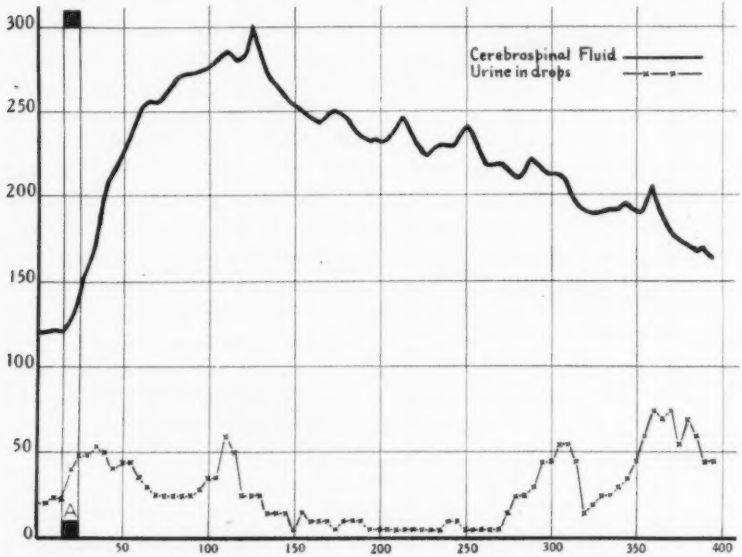

Chart 5. Experiment 12. Cat, weight 1985 grams. Ordinates represent millimeters of Ringer's solution and drops of urine (50 corresponding to 10 drops); abscissae represent time in minutes. During interval *A*, intravenous injection of 50 cc. of distilled water.

time on a gradual fall in pressure of the fluid occurred, with many peaks and depressions in the curve, but at the end of the period of observation the cerebrospinal fluid was still 40 mm. above its initial value. A distinct polyuria occurred immediately after the injection and again toward the end of the experimental period, the increases in urinary output determining possibly the rate of recovery.

The increase in the pressure of the cerebrospinal fluid, caused by the intravenous injection of distilled water, must be considered, then,

as being an invariable, outspoken and decisive reaction. In our experiments, the carotid systolic pressure was raised slightly by the introduction of the relatively large bulk of water—a finding first made by Magendie in 1838. Brachial venous pressure, taken as typical of the pressure in a systemic vein, may be considered to increase during the interval of injection; this increase seems to be in direct proportion to the degree of increase in the pressure of the cerebrospinal fluid. Thus in chart 3, in which a moderate rise in cerebrospinal fluid is recorded, a very small and momentary increase in brachial venous pressure is shown; in chart 4 a much more striking though temporary augmentation of both pressures is illustrated. Quantitatively and relatively, the increase in brachial venous pressure is in no sense proportionate to the increase in the pressure of the cerebrospinal fluid; the venous pressures quickly return to normal levels, long before subsidence of the augmented cerebrospinal fluid pressure is indicated.

Foley and Putnam (10) reported, in confirmation of the results of Weed and McKibben, that 50 cc. of "water given intravenously caused a marked and sustained rise of the cerebrospinal fluid pressure (100 mm.) which an hour after the injection was still at its height." On the other hand, their results with the hypotonic solution given intraduodenally were "not nearly so striking." The increase in the cerebrospinal fluid pressure under these conditions was not well sustained and within an hour normal readings were obtained. Foley and Putnam stated however that "water seems somewhat more effective when it follows by several hours the ingestion of a hypertonic solution."

Ebaugh and Stevenson (8) had their patient (an adult man) drink 4000 cc. of water in 75 minutes. A gradual increase in the intracranial pressure resulted showing as a rise of 20 mm. of water in the tambour—a minimal indication of the alteration in pressure.

HYPERTONIC SOLUTIONS

It has already been demonstrated that the intravenous injection of strongly hypertonic solutions causes a profound fall in the pressure of the cerebrospinal fluid and an associated decrease in the volume of the brain (Weed and McKibben (29), (30), Cushing and Foley (5), Sachs and Belcher (22), Foley and Putnam (10), Ebaugh and Stevenson (8), Sachs and Malone (23)). In the present series of experiments the earlier findings have been repeatedly confirmed; data regarding the systemic vascular alterations have also been obtained. The changes in

pressure of the cerebrospinal fluid following the injection of strongly hypertonic solutions are of greatest interest for it is with these solutions that negative pressures within the cranium have been obtained.

For the hypertonic injections in this series of experiments use has been made usually of a 30 per cent solution of sodium chloride in water—a solution of which the osmotic pressure is very great. In addition many experiments have been carried out with the intravenous injection of a so-called "concentrated Ringer's solution" in which the sodium, potassium and calcium salts are combined in the proportion found in Ringer's solution. This concentrated solution has been made up as follows:

NaCl	18.0 grams.
KCl	0.84 grams.
$CaCl_2$	0.5 grams.
H_2O to	100.0 cc.

The use of this concentrated Ringer's solution has yielded depressions of the cerebrospinal fluid in every way similar to those following the injection of strongly hypertonic solutions of the common sodium salts but with similar dosages, the depressions, as might be expected, have not been as great. In these experiments, however, the solution has not been found to cause the death of an animal; the intravenous injection of pure sodium chloride, even when given with great care, at times causes the death of the experimental animals. But in a normal, healthy animal under a proper degree of anesthesia the injection is seldom fatal if the administration is not too rapid.

The results of a typical experiment of this type in which an intravenous injection of 12 cc. of a 30 per cent solution of sodium chloride was administered, are graphically given in chart 6. The chart shows a control period of 10 minutes, during which the cerebrospinal fluid was constant at a level slightly above 105 mm., the brachial venous pressure around 80 mm. of Ringer's and the carotid systolic pressure fluctuated about 135 mm. of mercury. The sodium chloride solution was given at a rate of 2 cc. per minute over an interval of 6 minutes; no disturbances of respiration were noted during this period. The cerebrospinal fluid pressure rose as soon as the injection was begun, the rise occurring during the first 5 minutes, the peak being at 179 mm., but during the last minute of the injection-period it declined slightly. The brachial venous pressure receded slightly during the first minute of the injection, but it then rapidly mounted, attaining a height of

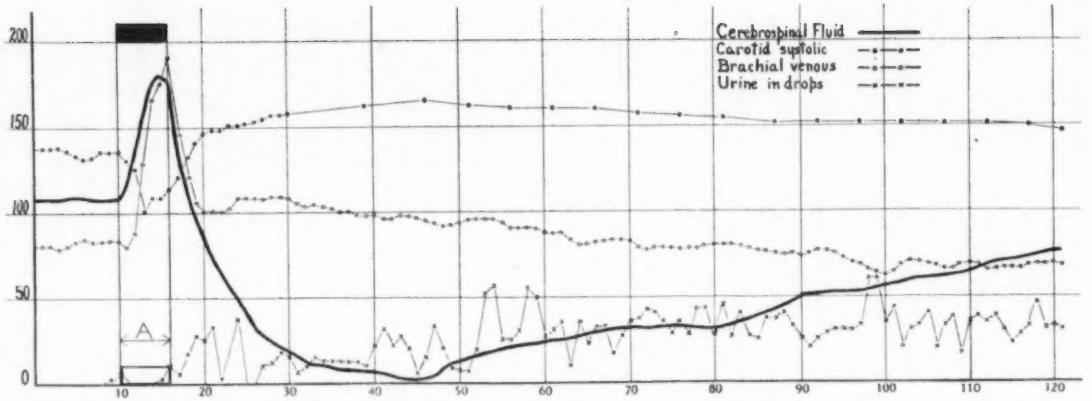

Chart 6. Experiment 50. Cat, weight 3000 grams. Ordinates represent millimeters of Ringer's solution or mercury (carotid pressure) and drops of urine (50 corresponding to 20 drops); abscissae represent time in minutes. During interval *A*, intravenous injection of 12 cc. 30 per cent solution of sodium chloride.

190 mm. at the end of the interval and exceeding the pressure of the cerebrospinal fluid. For the first half of this period of injection the carotid pressure fell (from 135 to 100 mm. Hg.); a slight recovery occurred during the latter half of the period. Urinary output was not increased during the interval of injection.

Immediately following the completion of the injection, both the brachial venous and the cerebrospinal fluid pressures fell rapidly, the readings of the latter pressure, however, being in every case lower than those of the former. The brachial venous pressure on returning to the 100 mm. level in 4 minutes, no longer fell at its previous rate but with a few slow fluctuations gradually declined to its initial level and then maintained a slightly decreased level until the end of the experiment. The cerebrospinal fluid, on the other hand, continued to drop in an even curve reaching its point of maximum depression at plus 1 mm. 28 minutes after completion of injection. This degree of extreme depression of the fluid was not maintained but gradual recovery took place, the fluid pressure slowly mounting to 75 mm. at the end of the record, exceeding in the last 9 minutes the brachial venous pressure. During this observation period the carotid systolic pressure rose slowly, reaching its maximum reading of 164 mm. Hg. at the same time as that of the greatest depression of the cerebrospinal fluid. This pressure then very slowly fell throughout the remainder of the experiment, the final value being 146 mm. Hg. The urinary output increased markedly after the injection, the polyuria being maintained throughout, though showing irregularities.

Such vascular reactions as those presented in chart 6 may be considered to be quite typical of the effect of the intravenous injection of hypertonic solutions. Many variations in the pressure effects during the period of injection have been observed; these are not only quantitatively but qualitatively different in the individual animals. A drop in the initial pressure of the cerebrospinal fluid immediately after the starting of the injection occurred in about half of the experiments; this was usually followed by a sharp rise, while the brachial venous pressure exhibited the marked rise throughout. In chart 7 an extreme increase in brachial venous pressure from 112 mm. to 219 mm. during the period of injection is recorded, while the pressure of the cerebrospinal fluid, not changing in the first minute of injection, rose from 127 mm. to 144 mm. only. The brachial venous pressure fell rapidly after the completion of injection and maintained throughout the 80 minutes of the record an irregular level somewhat below its initial normal reading. The

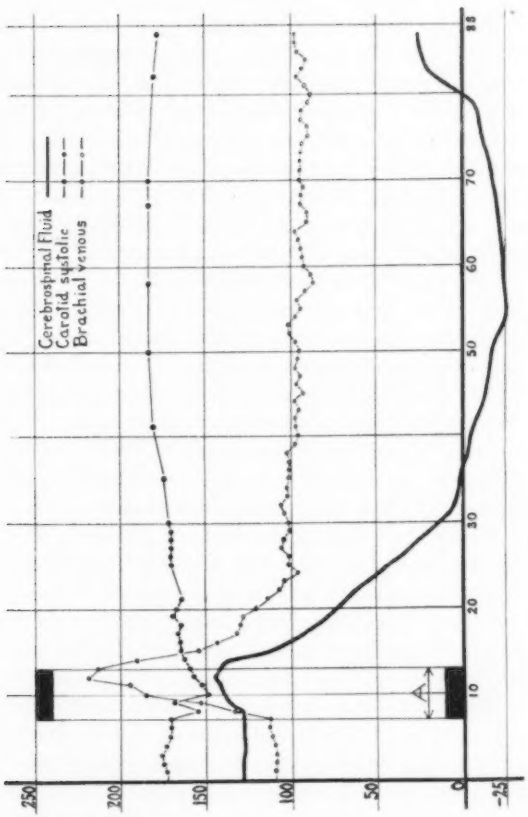

Chart 7. Experiment 27. Cat, weight 3150 grams. Ordinates represent millimeters of Ringer's solution or mercury (carotid pressure); abscissae represent time in minutes. During interval *A*, intravenous injection of 10.2 cc. of 30 per cent solution of sodium chloride.

fall in cerebrospinal fluid pressure was accomplished more slowly than in the animal represented in chart 6, the minimal reading of minus 25 mm. being obtained 41 minutes after the cessation of injection. Recovery of the cerebrospinal fluid pressure was quite slow for a few moments but toward the end of the record a sharp upcurve is shown. Carotid systolic pressure shows in the chart a gradual augmentation for some minutes, with a slight decline following. The record (chart 7) is included here to show that the change from positive to negative and again to positive pressures in the cerebrospinal fluid is not accompanied by significant changes in the systemic arterial or venous pressure.

In both charts 6 and 7 there is exhibited a tendency of the cerebrospinal fluid to return to its normal pressure levels, but the recovery is not complete in either case. A considerable number of cats was studied in regard to recovery from the depression of the cerebrospinal fluid pressure; the results indicate an extreme variation. In one of the earlier observations made under luminal sodium, a complete recovery of the pressure of the cerebrospinal fluid was observed in a 2-hour interval; in chart 8 also a complete recovery with reëstablishment of exactly the initial pressure readings is illustrated. In this animal the fall in the pressure of the cerebrospinal fluid was not extreme (from 143 to 90 mm.); the sustained lowering of the venous pressure suggests that its influence, as also that of the carotid pressure, in the restoration of normal cerebrospinal fluid pressure is not marked. For in spite of a gradual fall in the carotid pressure and the maintenance of a low level of brachial venous pressure the cerebrospinal fluid returned to its normal level and was maintained. Such cases as the two mentioned are, however, rare; examination of all the records from this standpoint indicates that usually recovery to a point of low positive pressure is exhibited in the 2-hour interval; likewise after 4 hours of experimentation the pressures are in a large majority of cases not yet returned to normal. In the longer periods (up to 7 hours) recovery is frequently but not invariably attained. The observations of Foley and Putnam (10) upon this recovery of pressure and of Sachs and Malone (23) on the persistence of shrunken brains over several hours are in accord with these ideas of a very slow recovery from the effects of the hypertonic solutions. Study of the rate of urine output in these cases has suggested an interesting relationship between the cessation of the marked polyuria and the beginning of a real recovery period; the findings in this regard have not led to any rule but have left a general impression of the relationship. In two experiments the renal vessels were ligated

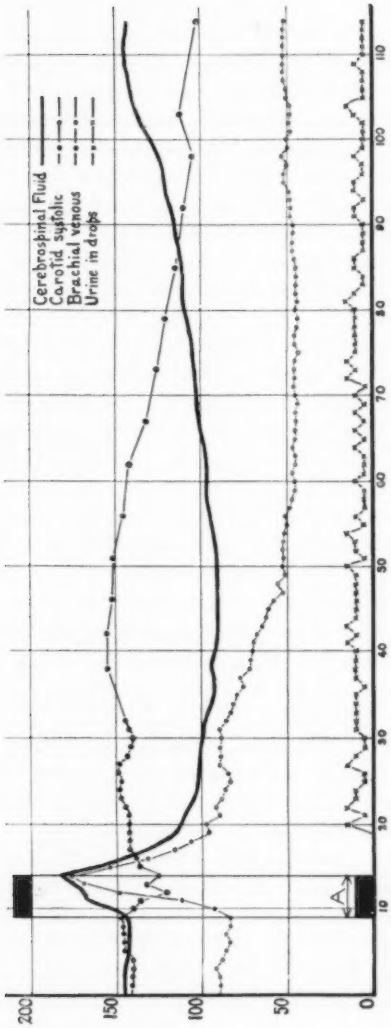

Chart 8. Experiment 45. Cat, weight 2800 grams. Ordinates represent millimeters of Ringer's solution or mercury (carotid pressure) and drops of urine (50 corresponding to 10 drops); abscissae represent time in minutes. During interval *A*, intravenous injection of 15 cc. "concentrated Ringer's solution."

on both sides before the injection of hypertonic saline was given; the lowering of the pressure of the cerebrospinal fluid seemed more prolonged than usual. The marked individual differences in reaction are however great enough to forbid more exact expression of this relationship without further evidence.

Quite exceptional in our experience has been the occurrence of a definite rebound in the pressure of the cerebrospinal fluid—a rise to a point beyond the initial readings. This occurred once in our experiments with animals under urethane; the terminal rise in this case might well have been due to the influence of the anesthetic. But also in a few other cases such a rebound has been noted—a phenomenon also observed by Ebaugh and Stevenson after administration of salt to their epileptic patient.

Several other phases of the reaction of animals to hypertonic solutions have been studied. Early in 1920 the results of experiments in which the effects of ingestion of strong saline solutions in the cat were studied, led to the belief that inconstancy of physiological reaction followed unless the salt was introduced elsewhere in the intestinal canal than in the stomach. In several of the etherized cats, to which concentrated salines were given by stomach tube, no effect upon the cerebrospinal fluid pressure was observed; subsequently at autopsy the stomach was found filled with the fluid. When the hypertonic solution was introduced into the alimentary tract below the stomach, prompt reduction of the cerebrospinal fluid pressure occurred. Foley and Putnam (10) have, however, already pointed out the essential physiological effects of such solutions; our results may be taken to be of the same positive character.

In several experiments we have computed the alterations in pulse rate and diastolic pressure which are effected by the intravenous injection of strongly hypertonic solutions of sodium chloride. In general it may be said that the diastolic pressures show no essential difference in reaction than do the systolic. Chart 9 is included here to show the pulse alterations in a cat in which a small dose of 30 per cent sodium chloride (5 cc.) was injected rapidly in 1 minute; the chart is prepared by plotting 30 second readings. During the period of injection the pulse rate rose slightly and then fell, to rebound quickly to a somewhat higher level. With slight decrease, this heightened pulse rate was maintained throughout the record. The great disturbance in carotid arterial pressure occasioned by this rapid injection of a small amount of the solution is well shown in the chart, as is the great increase in

brachial venous pressure, unaccompanied by a rise in the pressure of the cerebrospinal fluid which shows a prolonged fall throughout the period of observation.

The data from all of the experiments in this series of cats have been analyzed. In 56 animals of an average weight of 2778 grams, an average reduction of 88.1 mm. in the pressure of the cerebrospinal fluid was caused by an average intravenous injection of 1.155 grams of sodium chloride per kilogram of body weight. The injection of 1

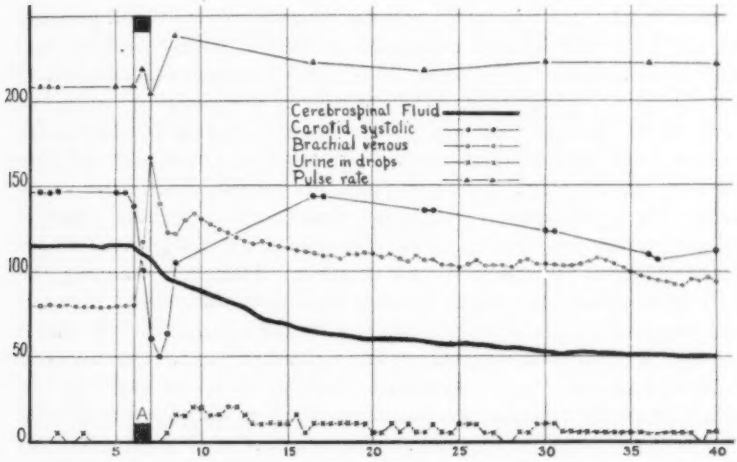

Chart 9. Experiment 63. Cat, weight 2800 grams. Ordinates represent millimeters of Ringer's solution or mercury (carotid pressure), pulse rate, and drops of urine (50 corresponding to 10 drops); abscissae represent time in minutes. During interval *A*, intravenous injection of 5 cc. of 30 per cent solution of sodium chloride.

gram of sodium chloride per kilogram of animal caused an average reduction of the pressure of the cerebrospinal fluid of 77.45 mm.

The general systemic effects of the intravenous injection of hypertonic solutions, apart from the pressure of the cerebrospinal fluid, have been investigated by many workers. The first contribution to the subject was apparently that made by Guttman (12), who showed that 5 grams of sodium chloride injected as a 20 per cent solution caused the death of rabbits. Klikowicz (18) determined the chemical changes in the blood occurring after intravenous injection of sodium

chloride in dogs. A marked increase (roughly 50 per cent) in the chlorides of both whole blood and serum was found in specimens obtained 2 minutes after the injection; after 1 hour the chloride content was still high. Heinecke (14) studied the reactions of frogs to immersion in strongly hypertonic solutions of sodium chloride; prolonged or repeated immersion led to convulsions and death. In an investigation of the effect of sodium chloride in large doses upon intravascular clotting, Silbermann (26) found that the intravenous injection of 4 to 12 grams of sodium chloride, inevitably led to convulsions and death in the rabbit. Heinz (15) was apparently the first to observe blood pressure changes after injection of hypertonic sodium chloride solutions; arterial pressure after injection of 20 cc. of a concentrated solution of sodium chloride (5 per cent ?) was found to fall gradually until the death of the animal. Münzer (20) recorded a similar fall in carotid pressure from the beginning of the injection of a 10 per cent solution of sodium chloride: this decrease in pressure continued to death, rising during the convulsions. The lethal toxic dose of sodium chloride was determined by Münzer to be 3.72 grams per kilogram of bodyweight, if given as a 10 per cent solution. Selig (24) studying the effect of inorganic salts upon lowered blood pressure, found that the intravenous injection of 1.8 per cent solution of sodium chloride raised the pressure more efficiently than a like injection of an isotonic solution. Similar conclusions that hypertonic solutions (5 to 10 per cent) of sodium chloride in small doses were capable of raising the arterial pressures in exsanguinated animals were reached by Retzlaff (21). In an important study of this question, Seppä (25) found that the injection of a hypertonic solution (sodium chloride) caused a sudden increase in the arterial pressure, due apparently to contraction of the blood vessels; this was followed in 30 to 70 seconds by a fall to the initial level. Then more slowly there occurred a secondary increase in arterial pressure due to the fluid flowing from the tissues into the blood stream, with later a gradual recession from this high point of pressure. A difference in lethal dosage of such solutions of sodium chloride was noted by Seppä in intact and exsanguinated animals; the lethal dosage varied from 1.70 to 1.95 grams per kilogram for the intact animal and 0.198 to 0.296 gram per kilogram of body weight for the bled. On rapid injection (1 cc. per minute) of 26.4 per cent solution of sodium chloride, Seppä ascertained the lethal dosage to be 1.95 grams per kilogram of animal—a lower value than that of previous investigators. He attributed the lowering of the lethal amount

of salt to the strength of the injection-fluid and the rapidity with which the injections were given.

The findings of Seppä with the stronger solutions of sodium chloride are quite comparable to the conditions holding in our experiments. We have kept well within the lethal dose of the sodium chloride in practically all cases; the fatalities which have occurred have been due to the rapidity of injection in all probability rather than to the absolute quantity of sodium chloride introduced. This interpretation is in accord with that given by Sachs and Malone (23). The arterial reactions presented here are quite similar to the records given by Seppä (25) and to the one graph in Foley and Putnam's (10) paper. The profound changes in brachial venous pressure and the less marked, though still significant, change in arterial pressure, during and after the period of injection indicate that in addition to the extreme alteration in pressures of the cerebrospinal fluid, there are systemic factors to be considered in the physiological interpretation of the findings.

DISCUSSION

In the foregoing sections of this report the changes in the systemic vascular system and in the cerebrospinal fluid as effected by the introduction of solutions of various concentration, have been detailed. Under experimental conditions which yielded uniform readings of all the pressures recorded, the intravenous injection of a large quantity of Ringer's solution caused a marked but wholly temporary rise in the pressure of the cerebrospinal fluid followed by a fall within a few minutes to normal levels. The changes in brachial venous pressure were in the same direction but of different magnitude, while depression of the arterial pressure occurred during the injection, with later recovery. The marked increase in the pressure of the cerebrospinal fluid, due to the intravenous injection of distilled water, was accompanied by changes in the brachial venous pressure in the same direction but not of the same magnitude, normal pressures in the veins being restored long before the cerebrospinal fluid returned to its initial level. The arterial changes were slight increases during and following the period of injection. With the intravenous injection of strongly hypertonic solutions, there were shown greater separations between the various pressures. Brachial venous pressure always mounted tremendously during the injection period; in about half of the cases the cerebrospinal pressure showed an initial drop before undergoing its typical elevation while in the other cases it rose immediately. A fall

in carotid pressure usually marked the period of injection. The profound depression of the cerebrospinal fluid pressure following the injection of such strong salt solutions could not be directly compared to the ultimate changes in the brachial venous pressure; this latter pressure was maintained at a level not markedly different from the initial readings. The slight lasting changes in arterial pressure were of relatively minor importance.

Consideration of all of the controlled factors in these experiments leads one to the view that alterations in the pressures of the cerebrospinal fluid can be brought about and maintained independently of the systemic vascular pressures. The most striking similarity in curves of pressures is that between the cerebrospinal fluid and the brachial vein; after the injection of Ringer's solution or of distilled water they exhibit somewhat the same alterations, differing not merely in magnitude but in duration. But this similarity of reaction disappears when one follows the curves of the two pressures as influenced by the intravenous injection of the hypertonic solutions. Brachial venous pressure invariably rises during the period of injection; in half of the cases the cerebrospinal fluid shows a sharp drop before the increase, if the increase does occur. But after the completion of the injection, the brachial venous pressure falls rapidly until it comes within the limits of its initial level while the cerebrospinal fluid pressure falls to far lower readings and frequently gives negative values of considerable extent. This divorcing of the two pressures has been a constant finding and is not only of immediate significance in the present problem but will be shown to have importance when the pressure of the cerebrospinal fluid is discussed in relation not only to systemic but to intracranial venous pressure. Brachial venous pressures in the control-periods have been lower usually by many millimeters than the pressure of the cerebrospinal fluid; this relationship holds after the injection of isotonic and hypotonic solutions but is reversed when hypertonic solutions are given.

The variability of the initial reaction of the cerebrospinal fluid at the onset of the injections of the cerebrospinal fluid suggests interesting speculations. The extraordinary rise in the brachial venous pressure during the whole period of injection might be held in part responsible for the rise of the fluid pressure; this increase in venous pressure could well overcome any tendency of the fluid to fall in pressure at this time in the injection-period. In rare instances, however, the pressure of the cerebrospinal fluid increases in the first minute of the

injection period before any rise in brachial venous pressure occurs. It seems, however, more than likely that the correct explanation of the initial fall in cerebrospinal fluid pressure is largely related to the sudden drop in arterial pressure; this decrease in arterial pressure may be balanced or overcome by the rapidly increasing venous pressure. Such an explanation involving the mechanics of the pressure-relations within the cranium seems preferable to one depending upon the assumption that the increased osmotic pressure of the blood affects so quickly the pressure of the cerebrospinal fluid.

In the final analysis the changes occurring in the pressure of the cerebrospinal fluid following the intravenous injection of solutions of various concentration must find their explanation in the alteration of the osmotic pressure of the circulating blood. The injection of a relatively large volume of Ringer's solution, isotonic with the blood, is followed merely by a short enduring rise of the cerebrospinal fluid pressure which subsides in approximately the same time-interval required for the pressure-changes effected by the hypotonic and hypertonic solutions to reach their maxima. For, as noted, the usual time for maximal reaction of the pressure of the cerebrospinal fluid is between 25 and 35 minutes after the end of the intravenous injection; rarely the maximal reaction is noted within 20 minutes and somewhat more frequently it is prolonged to one hour. During this interval the organism is attempting to compensate for alteration in the volume and in the salt-content of the blood. With the Ringer's solution the sole compensation is one for increased fluid-volume within the vascular system; this compensation, if judged by the time of return of the cerebrospinal fluid pressure to normal, is rapidly achieved. When, however, not only the volume of the blood is increased but its salt-content relatively diminished as by the intravenous injection of distilled water, two processes of adjustment proceed. The blood tends to reëstablish its normal salt-content by passage of water into the tissues and possibly into certain of the body fluids, and by attraction of salts from these places; and it also tends to compensate further for the increased volume of fluid by alteration of the vascular bed. The passage of fluid from blood vessel to tissue in the central nervous system may be assumed to be shown by the rise of the pressure of the cerebrospinal fluid and by the increase in brain bulk; the heightened arterial and venous pressures may be taken as a rough index of the increased vascular volume. The return of these vascular pressures to normal levels, while the pressure of the cerebrospinal fluid remains high,

would indicate the completion of certain phases of the process of fluid-passage into the tissues. In the phenomena attendant upon the injection of hypertonic solutions, there exist similar phases in the reaction. The increased volume of the blood, as shown by the heightened blood pressure, is due largely to the passage into the blood stream of fluid from the tissues and possibly from certain body fluids and not to the initial volume of fluid injected. This withdrawal of the tissue-fluid is indicated in the nervous system by the decrease in brain volume and in pressure of the cerebrospinal fluid. In all three of these types of reaction to the hypotonic, isotonic and hypertonic solutions, there results an increase in the volume of the circulating blood; with the first two solutions the vascular increase is immediate and due to the injection itself, while with the third the increase is slow and is due to the tissue-fluid passing into the blood stream. Of these changes the pressures recorded give some indication but there is need also for determinations of the fluid-volumes as well as the pressures. During all of these processes the excretion of fluid and salt through the urine is active, for as quickly as possible the organism attempts to rid itself of the increased salt or fluid.

Thus far it has not been possible to demonstrate with certainty whether, following the intravenous injection of the hypotonic solution, there is an increase in quantity of the cerebrospinal fluid nor has it been possible to determine an additional absorption of the fluid due to injection of a hypertonic solution. The evidence is clear that the alteration in osmotic pressure of the blood changes the size of the brain, the large brain resulting from the hypotonic solution and the small brain from the hypertonic. Also, as Weed and McKibben reported, the introduction of a foreign solution into the subarachnoid space coincident with the reduction of cerebrospinal fluid pressure by hypertonic salt, gave evidence of a penetration of the foreign solution into the brain substance in a manner markedly different from that occurring normally. And Foley and Putnam (10) presented suggestive evidence that after the injection of a hypertonic solution, a new ratio between the rate of secretion and absorption of the cerebrospinal fluid became established. The final elucidation of this phase of the problem will require additional work before absolute evidence is acquired.

CONCLUSIONS

1. The intravenous injection of relatively large amounts of Ringer's solution causes a temporary rise in the pressure of the cerebrospinal fluid and in the brachial venous pressure; both quickly return to normal levels. Arterial pressure is usually reduced during the period of injection and remains at a slightly lower level than that shown initially.

2. The intravenous injection of a hypotonic solution (distilled water) causes a prolonged increase in the pressure of the cerebrospinal fluid. This increase in pressure is accompanied by an increase in brachial venous pressure of far smaller degree and of shorter duration. Arterial pressure rises slightly in response to such injections.

3. The intravenous injection of strongly hypertonic solutions causes a prolonged and profound fall in the pressure of the cerebrospinal fluid preceded usually by a sharp rise. The brachial venous pressure increases markedly during the period of injection and then falls rapidly to maintain a new level, usually slightly below the normal. Arterial pressure is lowered during the period of injection but recovers to a level somewhat higher than the initial.

4. Cerebrospinal fluid pressure is invariably higher than that of the brachial vein, except after the intravenous injection of strongly hypertonic solutions.

5. The changes in cerebrospinal fluid pressure induced by the intravenous injection of solutions of various concentrations seem to be independent of the changes in the systemic arterial or venous pressures.

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THE CEREBROSPINAL FLUID IN RELATION TO THE BONY ENCASEMENT OF THE CENTRAL NERVOUS SYSTEM AS A RIGID CONTAINER

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Since the time of Alexander Monro the younger (13), practically all workers on the physiology of the intracranial contents have accepted the idea that the central nervous system is enclosed in a rigid system of bony coverings which serve as a closed box of fixed volume. The general conception has with the more modern advances in knowledge been modified greatly since its original presentation but the essential phases of the hypothesis have not been questioned.

Anatomically, the cranium possesses on gross inspection the accepted characters of a rigid container, with the dura mater everywhere tightly applied to the inner surface of the skull. In the spinal region, however, the similarity of the bony coverings to a "closed box" is by no means so simple of explanation. For though the vertebral bodies and arches form a rigid channel, bridged by the segmental ligaments, the dura does not closely hug the inner surfaces of these bony coverings. The epidural space intervening between bone and dura contains in the mammals an abundant venous plexus in the midst of fatty areolar tissue. Here then are anatomical structures which do not possess sufficient fibrous bundles to maintain satisfactory connection with the vertebrae: the question immediately arises as to whether on evacuation of the spinal dural contents the dura would remain in position due to the creation of a partial vacuum in the epidural tissue or whether the veins would dilate sufficiently to permit collapse of the dura. In the latter case the vertebral channel could not be assumed to constitute a "closed box" for the central nervous system. Likewise the occipito-atlantoid ligament possesses sufficient elasticity to permit transmission of the pulsations of the cerebrospinal fluid—a phenomenon first noted by Magendie and since observed by many workers.

But in addition to these rather obvious anatomical questions regarding the rigidity of the bony coverings of the nervous system still other aspects of the general problem are related to the possibility of escape of cerebrospinal fluid around the cranial and segmental spinal nerves. That such perineural channels exist has been demonstrated by the injection-method (11), (16) but the physiological significance of the structures is largely unknown. If retrograde passage of fluid into the central nervous system could occur along these channels, the closed character of the coverings of the central nervous system could not be held to be correct; modification of the entire view of a fixed total of cranial contents would have to be effected.

These factors (epidural tissue, occipito-atlantoid ligament and perineural spaces) seem to have but little influence on the maintenance of the rigid character of the bony coverings of the central nervous system when consideration is given to the frequent production of negative pressures of the cerebrospinal fluid as reported by Weed and McKibben (18). This finding of negative pressures in the fluid immediately argued strongly for the "closed box" conception of the cranial and vertebral channels and the modification of the doctrine suggested by them was based on the idea that within demonstrable physiological limits the central nervous system is enclosed in a rigid container. For it is obvious that had the coverings of the nervous system not been maintained rigidly in position collapse of the meningeal spaces would have followed the intravenous injection of strongly hypertonic solutions and the pressure of the cerebrospinal fluid would have remained positive.

Recently other experiments were undertaken for the purpose of gathering additional data relating to the cranium as a "closed box:" to make use of the power of strongly hypertonic solutions on intravenous injection to obtain an idea of the physiological limits within which the cranium serves as a rigid container. While the mere production of negative values in the cerebrospinal fluid might be considered physiological proof of this function of the bony coverings of the nervous system additional support to the conception was thought possible by proper experimental procedures. To this end, observations were carried out on animals in which in one series the bony calvarium on one side was largely removed without opening the dura and in the other series a smaller bony opening was subsequently sealed, restoring the intact cranium. In the two types of experiment, then, the central nervous system remained rigidly protected against expansion outward by the unopened inelastic dura; collapse of the dura in the first series

could easily occur, while in the second type of experiment opening of the cranial cavity could at any time be effected by removal of the sealing mechanism. Under these conditions injections would, it was hoped, give information, by their effect upon the pressure of the cerebrospinal fluid, regarding the relation of the cranial vault to the rigid character of the bony coverings of the central nervous system.

METHODS OF INVESTIGATION: The technical procedures employed were devised to permit the recording of the pressure of the cerebrospinal fluid in animals in which the bony calvarium was opened. The animals (young adult cats) were anesthetized with ether and were placed on a suitable board lying on their left sides. Cannulae were introduced to give records of the carotid arterial pressure, brachial venous pressure and of the urine output in the manner described in the preceding paper (17). In two cases, in addition to the brachial venous pressure a cannula was inserted into the right internal jugular vein toward the cranium. The hypertonic salt solutions (30 per cent sodium chloride in distilled water) were injected from a burette directly into a brachial vein. The pressure of the cerebrospinal fluid was obtained as in the preceding experiments.

The opening of the cranial chamber required care for its success. In the first series of experiments the right temporal muscle was reflected and a trephine hole of 2 cm. made through the underlying bone. This opening was enlarged by the use of rongeurs so that a large proportion of the bone covering the right cerebral hemisphere was removed. Care was exercised so as to avoid any injury to the dura mater; for the success of the experiment an intact unopened dura was essential. All bleeding from the diploetic vessels was controlled by use of bone wax. Under these conditions, the pressure of the cerebrospinal fluid was obtained by insertion of a needle through the occipito-atlantoid ligament, and it was held necessary that the fluid should be clear and without blood staining.

In the second type of experiment, the procedure in its initial stages was identical but the trephine hole was either not extended or enlarged but little. With a blunt dissector the dura was widely freed from the skull and soft vaseline introduced to lessen subsequent adhesion of the two surfaces. The trephine hole was then filled with a hard vaseline and the opening closed by placing an appropriate glass slide over it, sealing the edges carefully with the vaseline. The pressure of the cerebrospinal fluid was then recorded in the usual way; here again a clear fluid was held essential for demonstrating that no injury to the meninges had been caused.

In some of the experiments the record of arterial pressure was omitted, as the recording of this pressure was not necessary for the physiological phenomenon to be tested.

EXPERIMENTAL FINDINGS: *With the bony skull on one side opened.* With the experimental procedures carried out as indicated (the bony skull on one side of the animal being opened widely without injury to the dura mater), the cerebrospinal fluid was found to have a normal pressure with the usual excursions in the manometer. While the preparation as made possessed an inelastic membrane preventing expansion outward, collapse inward was possible; under these circumstances negative pressures in the cerebrospinal fluid could not occur if these negative pressures were to be considered as being dependent upon the assumed character of the cranium as a rigid box.

Chart 1 gives a record of such an observation. As may be noted, the cerebrospinal fluid was recorded during the control period at 135 mm. while the brachial venous pressure was slightly above 60 mm. Three separate intravenous injections of a 30 per cent solution of sodium chloride were given to this young cat of 2100 grams; each injection was followed by a fall in the pressure of the fluid, the first of far greater magnitude than the subsequent ones. During the first injection (10 cc.) of the hypertonic solution, a sharp fall with quickly following rebound occurred; the subsequent drop in the pressure of the cerebrospinal fluid was somewhat slower than in the intact animal, a level being shortly reached at about 40 mm. Thirty minutes after the first injection a second of 8 cc. of 30 per cent sodium chloride was made; this caused a second fall in the pressure of the cerebrospinal fluid to a level at 25 mm. The third injection, given one-half hour after the second, provoked a very slight further reduction of the pressure of the cerebrospinal fluid to 20 mm. This was maintained with slight recovery throughout the remainder of the period of observation. Brachial venous pressure during the experiment showed typical reactions with marked abrupt rises during the periods of injection and gradual returns to somewhat higher levels than existed before the injection. The usual physiological response to the hypertonic solutions was shown in the urinary output.

The record presented in chart 1 may be taken as typical of the results in this series of experiments. The animals used were young cats of weight slightly in excess of 2 kilograms; in these, if the cranium be unopened, the production of a negative pressure of the cerebrospinal fluid by a single injection of 10 cc. of the 30 per cent solution of sodium

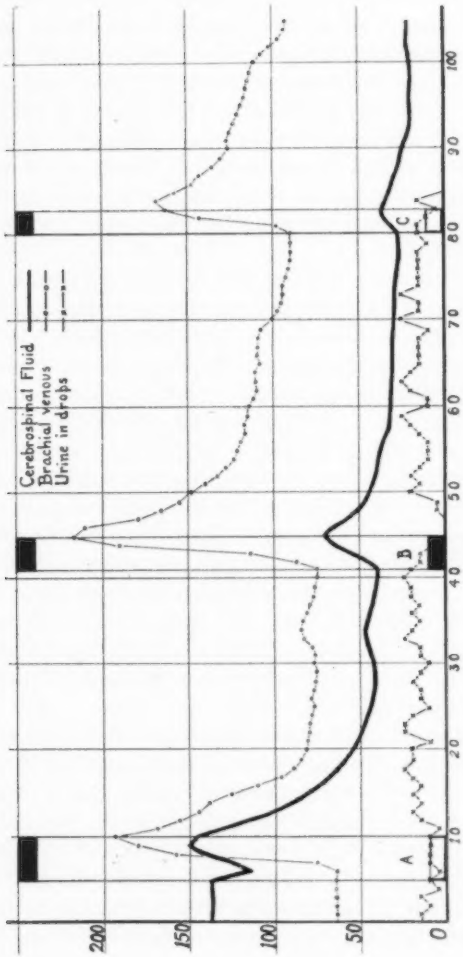

Chart 1. Experiment 89. Cat, weight 2100 grams. Ordinates represent millimeters of Ringer's solution and drops of urine (50 corresponding to 10 drops); abscissae represent time in minutes. During interval *A*, intravenous injection of 10 cc. of 30 per cent solution of sodium chloride; during interval *B*, intravenous injection of 8 cc. of same solution; during interval *C*, intravenous injection of 5 cc. of same solution. Right half of calvarium removed.

chloride was almost invariable. Repeated injections as given in the recorded experiment have not failed in the intact animal to give negative readings of the cerebrospinal fluid pressure. But in none of the observations of this type, in which the bony covering of one cerebral hemisphere was removed, have repeated injections of the hypertonic solution (even to great excess in dosage) produced negative pressures in the cerebrospinal fluid. One is led therefore to infer that the production of negative pressure of the cerebrospinal fluid is directly related to the intact cranial vault; the central nervous system, as judged by the results of this type of experiment, must therefore be considered as possessing, within tested physiological limits, a rigid covering—as lying within a "closed box."

Examination of the exposed outer surface of the dura in this group of experiments gave some indication of the effect of the injection of hypertonic solution. In the initial control periods the dura was normally tense and showed a rhythmic pulsation synchronous with the respiratory movements. During the period of injection it became rigidly tense, without visible pulsation; but with the reduction of cerebrospinal fluid pressure following, this tenseness rapidly disappeared. As the fluid pressure fell to its new reduced level, the dura became quite flaccid and fell away from the cranial defect; with repeated injections, it became somewhat more tense during each injection-interval (with the momentary increase of intracranial pressure) and then became more and more flaccid as the pressure of the cerebrospinal fluid fell. In the later stages, after repeated injections, the dura was loosely applied to the cortical convolutions, flaccid and markedly lower than the original cranial vault; for in such experiments, marked shrinkage of the brain tissue occurs (19), (4), (14), (8), (15).

It was soon obvious in these experiments that the ultimate level of the positive pressure of the cerebrospinal fluid was determined by the hydrostatic height of the brain tissue above the puncture-needle. Under the conditions of experimentation, the occipito-atlantoid puncture was made in the mid-sagittal line posteriorly, and the zero of the manometer was properly set at this level. As the animal lay on its left side the right half of the nervous system was above the level of the needle; the right cerebral hemisphere became the highest point of any portion of the nervous system. Measurements of this hydrostatic height of the right cerebral hemisphere above the needle were taken at intervals in each experiment; these measurements gradually decreased with the shrinkage of the brain. The positive pressure of

the cerebrospinal fluid was found to be always identical with or slightly greater than the hydrostatic height of the brain above the needle or zero point on the manometer; this indicated that the persisting positive pressure of the cerebrospinal fluid was directly determined by the height of the brain. Hence, with the dura collapsing upon the shrinking cerebral hemisphere, negative pressures of the cerebrospinal fluid were not obtained.

With opening and subsequent sealing of cranium. With the experimental data indicating that the unilateral removal of the bony vault of the cranium prevented the occurrence of negative pressures of the cerebrospinal fluid, the other series of observations, designed to give further evidence regarding the mechanism of a "closed box" as constituted by the cranium, was undertaken. In this second series, the skull on one side was opened, the intact dura widely freed from the cranium, and the cranial defect sealed with heavy vaseline and a glass slide. The nervous system was then enclosed by an inelastic membrane preventing expansion outward (dura); collapse inward was opposed by the sealing of the cranial opening. In such animals, therefore, appropriate intravenous injections of strongly hypertonic solutions could produce negative pressures of the cerebrospinal fluid while removal of the sealing mechanism would give immediately the desired experimental state of an opened cranium with a dura which could collapse inward. On this premise, conversion of a negative pressure of the cerebrospinal fluid to positive readings by release of the sealing mechanism would be interpreted to indicate that under the established physiological conditions the "closed box" character of the coverings of the nervous system were largely due to an intact cranial vault.

The correctness of this hypothesis is demonstrated in chart 2, which is compiled from the records obtained on a young adult cat of 2200 grams. The initial pressure of the cerebrospinal fluid (133-134 mm.) was maintained during the control period of 5 minutes, after which an intravenous injection of 10 cc. of a 30 per cent solution of sodium chloride was given. The pressure of the cerebrospinal fluid dropped at the beginning of the injection and then rebounded to a higher level, which was reached at the end of the injection-interval. Immediately there followed a rapid fall in the cerebrospinal fluid pressure, tapering off gradually as the pressure went below zero. A negative pressure of minus 8 mm. was attained 19 minutes after the end of the injection; at this time (before the subsequent rise in the cerebrospinal fluid pressure could occur) the cranium was opened by removal of the sealing device.

The pressure of the cerebrospinal fluid forthwith (as shown by chart 2) rose to a positive pressure of 20 mm., and this pressure was maintained throughout the period of observation (28 minutes longer). The findings in this experiment, typical of others in the series, can be explained only on the basis that an intact cranium is essential for the production of negative pressures in the cerebrospinal fluid.

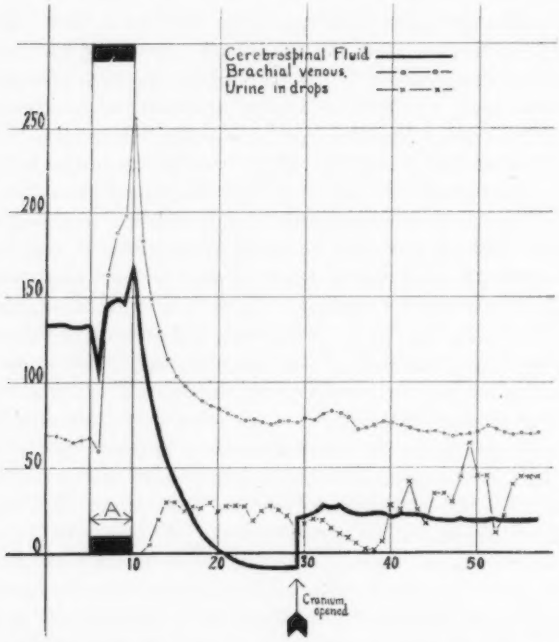

Chart 2. Experiment 52. Cat, weight 2200 grams. Ordinates represent millimeters of Ringer's solution and drops of urine (50 corresponding to 20 drops); abscissae represent time in minutes. During interval *A*, intravenous injection of 10 cc. of 30 per cent solution of sodium chloride. Cranium opened and subsequently sealed.

In chart 2 there are also recorded the brachial venous pressure and the urinary output. In this animal the brachial venous pressure fluctuated about a level slightly above 60 mm. of Ringer's solution; during the period of injection it first showed a slight drop and then a remarkable rise to 255 mm., far exceeding the pressure of the cerebro-

spinal fluid. After completion of the injection the brachial venous pressure fell rapidly to come to rest at a level of about 75 mm. Opening of the skull did not cause any change in this brachial venous tracing. The plotting of the urinary output indicates a typical polyuria following the injection and continuing throughout.

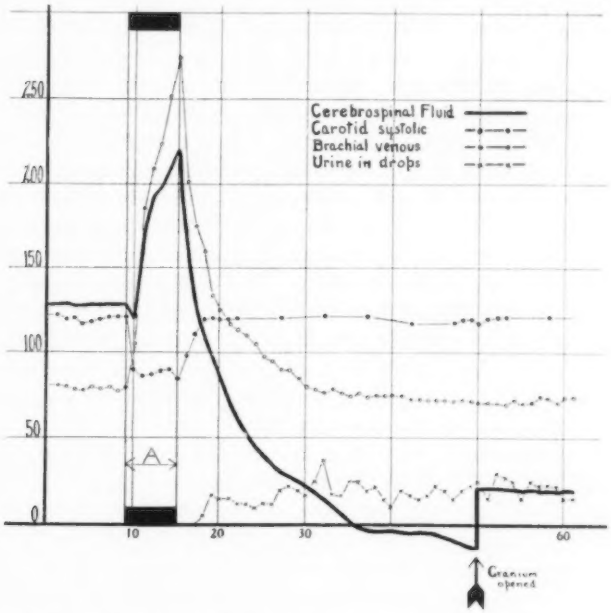

Chart 3. Experiment 80. Cat, weight 2200 grams. Ordinates represent millimeters of Ringer's solution or mercury (carotid pressure) and drops of urine (50 corresponding to 20 drops); abscissae represent time in minutes. During interval *A*, intravenous injection of 13.4 cc. of 30 per cent solution of sodium chloride. Cranium opened and subsequently sealed.

A result quite similar to this is given in chart 3, compiled from the records of a young adult cat of the same weight (2200 grams), but with the arterial pressure also included. In this, after an initial control period of 9 minutes during which the cerebrospinal fluid, the carotid systolic and brachial venous pressures showed normal levels, an intravenous injection of 13.4 cc. of 30 per cent solution of sodium chloride was administered. During the first minute of this interval the cerebrospinal

fluid pressure dropped slightly while the brachial venous pressure rose 25 mm. Throughout the rest of the injection both the brachial venous and the cerebrospinal fluid pressures rose markedly, the former outstripping the latter. The carotid systolic pressure dropped considerably during the first minute to a new level which was held during the remainder of the injection-interval. On completion of the injection the pressure of the cerebrospinal fluid fell at a rate slightly less than that shown in chart 2 but it came to have a reading of minus 13 mm. after 35 minutes. Meanwhile the carotid systolic pressure had reached and maintained a new level as had also the brachial venous. The cranium was suddenly opened at this time by removal of the sealing device; the pressure of the cerebrospinal fluid immediately rose from minus 13 mm. to plus 20 mm. No variation in brachial venous pressure was noted when this abrupt change in cerebrospinal fluid pressure was achieved; the carotid pressure, of which a continuous record was taken, showed no marked deviation. The cerebrospinal fluid pressure was maintained at this new level, practically identical with the measurement of the hydrostatic height of the cerebral hemisphere above the needle, with variations of 2 mm. or less in the readings. The former levels of the carotid systolic and brachial venous pressures were unaltered throughout the remainder of the record.

The striking results obtained on opening the cranium in these animals in which negative pressures of the cerebrospinal fluid were existent were invariable under the experimental conditions. At times, however, additional injections of the hypertonic solution were required to reduce the pressure of the cerebrospinal fluid to negative values; in these cases also the opening of the cranium promptly caused the pressure of the fluid to become positive. The record from one animal of this type is reproduced as chart 4, which includes a tracing of the pressure of the internal jugular vein taken toward the cranium. The occurrence of internal jugular veins in the cat was found to be quite variable and the reproduced record is from one of two experiments of the type in which reliable data could be obtained. The experimental procedure did not prove to be satisfactory, as the introduction of Ringer's solution through this cannula into the internal jugular invariably raised the pressure of the cerebrospinal fluid. It was apparently for that reason that four injections in this young adult cat of 2200 grams were required to produce the negative pressure. The chart shows, in addition to the typical responses already illustrated in previous charts, the marked correspondence between the jugular and brachial pressures throughout,

for after the initial control interval the two pressures were of almost exactly the same magnitude. The opening of the skull in this case caused practically no change in either brachial or jugular venous pressures even though the pressure of the cerebrospinal fluid rose from minus 5 mm. to plus 33 mm.

The evidence, then, compiled from this type of experiment, indicates that the pressure of the cerebrospinal fluid may be converted from

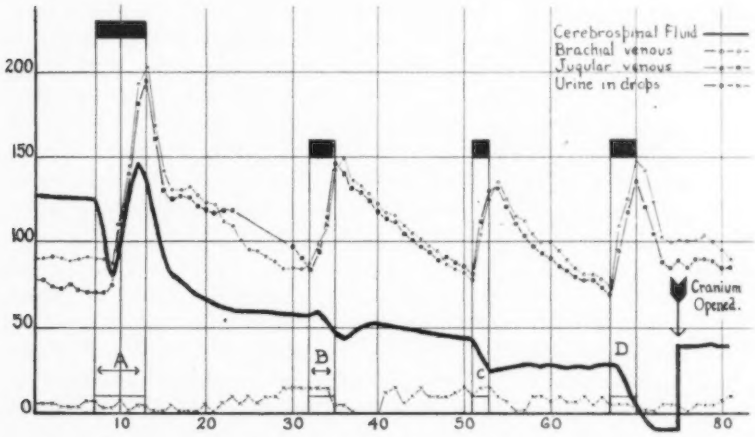

Chart 4. Experiment 60. Cat, weight 2200 grams. Ordinates represent millimeters of Ringer's solution and drops of urine (50 corresponding to 20 drops); abscissae represent time in minutes. During interval *A*, intravenous injection of 10 cc. of 30 per cent solution of sodium chloride; during interval *B*, 5 cc. of same solution; during interval *C*, 4 cc. of same solution; during interval *D*, 5 cc. of same solution. Cranium opened and subsequently sealed.

negative to positive values by opening of the cranium. It strongly substantiates the view that the coverings of the central nervous system form a rigid container within which the pressure of the cerebrospinal fluid may be reduced to negative values. When the integrity of the bony skull was destroyed by opening the cranium, negative pressures in the cerebrospinal fluid were not obtained.

DISCUSSION

The hypothesis that the quantity of blood circulating within the bony coverings of the central nervous system is at all times constant was first brought forth by Alexander Monro the younger (13) in 1783. The conception was based on the assumption that the substance of the brain like that of the other solids of the body is nearly incompressible and is "enclosed in a case of bone," assuring therefore the constancy of the blood-content. Monro's idea was developed by Kellie (10), who in 1824 performed supposedly critical investigations of the quantity of blood existing within the cerebral vessels of animals and of men frozen to death. He concluded that in the intact condition of the cranium the quantity of blood could not be altered in any way, but if the animal be trephined the intracranial blood could be largely removed by hemorrhage. Many clinical investigations followed Kellie's studies and were considered to substantiate this Monro-Kellie doctrine, which received wide acceptance through Abercrombie's (1) publications on apoplexy.

The original Monro-Kellie doctrine was evolved before knowlege of the cerebrospinal fluid was at all general, but with Magendie's studies of the fluid, modification of the doctrine became necessary. Burrows (3) was apparently the first to point out the importance of the cerebrospinal fluid as a factor in the intracranial contents and his general conception of the intracranial relationship is probably the best given by any of the earlier writers. The incompressible character of the brain substance was not emphasized by Burrows for he felt that any vacated space in the cranium could be replaced by "extravascular serum" (cerebrospinal fluid) or "resiliency of the cerebral substance under diminished pressure." He admitted that "the whole contents of the cranium, that is, the brain, the blood and this serum (cerebrospinal fluid) together, must be at all times nearly a constant quantity."

Many physiologists subjected the doctrine of a constant intracranial volume of blood to experimental tests and came to different conclusions with regard to the accuracy of the assumption. Kussmaul and Tenner (12) and also Donders (6) attempted, by direct observation through a cranial window, to secure evidence regarding the intracranial vascular volume; their methods, while more reliable than observations on dead animals, did not permit control of all the factors. The evidence presented by these workers hardly justified their conclusion of a variable

intracranial blood-volume, as pointed out by Leonard Hill (9). Hill, introducing more rigid methods of control, felt that "the volume of blood in the brain is in all physiological conditions but slightly variable." Dixon and Halliburton (5) studied the general problem of the Monro-Kellie doctrine in a somewhat different way, showing great variation in the intracranial pressures and the relations of the cerebrospinal fluid to the pressure in the torcular herophili. Their assertion that (p. 153) "the cranial contents cannot any longer be regarded as a fixed quantity without the power of expanding or contracting in volume" necessarily involved the modification of the doctrine; certainly their findings indicated that within the physiological limits established, variations in the pressures of both cerebrospinal fluid and cerebral venous blood could be effected without the exact correspondence in pressure-relationships given by Leonard Hill. This idea of variability in pressure-relationships has been of the greatest value in the more recent advances of knowledge regarding the cerebrospinal fluid.

The experimental alteration of the volume of the brain effected by Weed and McKibben (19) necessarily introduced another variable element into the considerations of the doctrine of the fixed contents of the cranial cavity. They stated that (p. 553) "the cranial cavity is relatively fixed in volume and is completely filled by brain, cerebrospinal fluid and blood; variations in any one of the three elements may occur, compensation being afforded by alteration in the volume of one or both of the remaining elements." This idea which is somewhat similar to that early advanced by Burrows (3) and in a way comparable to that of Dixon and Halliburton (5) was based largely on the necessary anatomical explanation of their findings in regard to the production of negative pressures of the cerebrospinal fluid and to the experimental alteration of the brain volume. The occurrence of negative pressures of the cerebrospinal fluid suggested strongly that the cranium was within the tested physiological limits a "closed box" while the experimental alteration of brain volume introduced the conception that the volume of the brain was not absolutely fixed but that within narrow limits physiological variation in its bulk occurred.

Becht (2) who has recently published upon certain phases of the problems of the cerebrospinal fluid, has accepted the doctrine of the closed cranium. He has stated one aspect of the doctrine as follows (p.12):—"It is well known that both brain and cord, because of their large water content, are practically incompressible; because of its bony structure the skull and neural canal are nearly undilatable to pressure

except at the membranes covering the foramina between the vertebrae. To the same degree that the the brain is incompressible and the skull undilatable by pressure are they lacking in elastic recoil when the pressure is removed."

It is apparent that the Monro-Kellie doctrine of fixed intracranial contents has been largely accepted by the workers in the field, though modified to meet the requirements of the later work. But it seems clear that practically none of the workers have determined accurately the anatomical mechanism which constitutes the closed rigid system, though physiological hypotheses have been developed largely upon its acceptance. Kellie (10) really subjected the conception to experimentation and his account of the recession of the brain from the skull in an animal which had been trephined (dura opened?) marked the initial demonstration of the function of the intact skull in the intracranial relationships. Ecker (7) also noted in a trephined animal a marked diminution in the size of the brain when the carotid arteries were divided. Such observations, in contrast to the findings in animals with intact cranial cavities gave experimental proof of this function of the bony cranium as a factor in the maintenance of the closed container for the central nervous system.

The experiments detailed in the foregoing sections of the communication represent an additional attempt to test the correctness of the hypothesis that the cranium and vertebral canal constitute a rigid and closed mechanism. The data obtained show that the integrity of the cranium is essential for the operation of the physical laws of the "closed box" as applied to the bony coverings of the nervous system. For with the bony covering of one hemisphere removed without disturbing the dura repeated injections of strongly hypertonic solutions fail to lower the pressure of the cerebrospinal fluid to negative values. And similarly, if the cranium be opened and subsequently sealed, the injection of the concentrated saline may then produce negative pressures in the cerebrospinal fluid; opening of the cranium by removal of the sealing device under these conditions causes the pressure of the cerebrospinal fluid to become positive. The level of the persisting positive pressure of the fluid in both cases is apparently determined by the hydrostatic height of the cerebral hemisphere above the puncture-needle.

Such evidence seems to place the conception of the "closed box" character of the bony coverings of the central nervous system on a somewhat firmer basis than that upon which it rested before. The mere obtaining of negative pressures within the subarachnoid space

demonstrated the essential truth of the statement, but the phenomenon might conceivably have been due to other factors than the mere rigidity of the cranial vault. It would now seem fair to assume that within the physiological limits tested, the cranium functions as a "closed box;" the fluid-pathways about the cranial and spinal nerves, the potential expansibility of the spinal epidural space and elasticity of the occipito-atlantoid ligament are factors whose importance it seems now safe to minimize. While in many ways modified to meet the more recent advances in our knowledge, the Monro-Kellie doctrine seems essentially sound; the view expressed by Abercrombie (1) some ninety years ago may, with but few reservations, be accepted (p. 300): "The cranium is a complete sphere of bone, which is exactly filled by its contents, the brain, and by which the brain is closely shut up from atmospheric pressure, and from all influence from without except what is communicated through the blood-vessels which enter it." If one introduces into this statement the idea of variability in the quantity of circulating blood and of the cerebrospinal fluid and the conception of variability in the volume of the brain, the conception will hold today.

SUMMARY AND CONCLUSIONS

1. Repeated intravenous injections of strongly hypertonic solutions fail to reduce the pressure of the cerebrospinal fluid to negative values in animals in which the bony skull over one cerebral hemisphere has been removed.

2. Negative pressures of the cerebrospinal fluid are obtained by intravenous injections of strongly hypertonic solutions in animals in which the opening through the skull has been subsequently sealed; under these experimental conditions opening of the cranium by removal of the sealing device causes an immediate rise in the pressure of the cerebrospinal fluid to positive readings.

3. These findings indicate that the bony coverings of the central nervous system constitute within tested physiological limits, inelastic and rigid containers; the ordinary physical laws of a "closed box" may therefore be applied to the cranium.

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INTRACRANIAL VENOUS PRESSURE AND CEREBROSPINAL FLUID PRESSURE AS AFFECTED BY THE INTRAVENOUS INJECTION OF SOLUTIONS OF VARIOUS CONCENTRATIONS

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The relationship existing between the pressure of the cerebrospinal fluid and the pressure in the dural sinuses has since the publication of Leonard Hill (7) in 1896 been the subject of important studies. The conception of equality of pressures in the two systems brought forward by Hill has been attacked in the last decade; the more recent investigations indicate that variation in the pressure in the torcular herophili may cause change in the pressure of the cerebrospinal fluid, while according to other workers alteration in the pressure of the cerebrospinal fluid may not influence the pressure in the dural sinuses. The technical procedures employed have in the main been identical but the relative difficulties of control have introduced elements which have apparently affected the correctness of many of the statements of general laws.

The marked alterations in the pressure of the cerebrospinal fluid effected by the intravenous injection of solutions of various concentrations (12) have been thought to afford a somewhat novel basis of attack for the general anatomical and physiological problems concerned in the intracranial pressure relationships. In the initial paper in this series (10) the authors have attempted to show that such alterations in the pressure of the cerebrospinal fluid were brought about without essential change in the systemic vascular pressures and in the second paper (11) they have presented additional proof of the correctness of the Monro-Kellie doctrine that within definite limits the intracranial contents are of fixed volume. For it was held essential, before investigating the general question of intracranial pressure relationships, to ascertain whether the skull and vertebral canal did function, within the limits of the physiological reactions studied, as a "closed

box," because it is upon this conception that any discussion of the relation of the cerebrospinal fluid pressure to the intracranial venous pressure must depend. With positive evidence that the cranium and vertebral canal do constitute a rigid system within which are contained the central nervous system and its coverings, it became possible to investigate these intracranial pressures, making use of the intravenous injection of solutions of various concentrations to study the relationship of the pressure of the cerebrospinal fluid to the intracranial and systemic vascular pressures. It is with this problem then that the present communication will deal.

Methods of investigation. The general technical procedures followed in this study were the same as those described in the initial paper of this series (10) with but few additions and modifications. Both cats and dogs were used, but the greater surety of the experimental procedure on dogs led to their use for all important observations. Ether was employed as the sole anesthetic, for in our hands more constant pressures could be obtained under it than with any other anesthetic. As in the foregoing studies, the ether was administered through a Woulfe bottle connected with a tracheal cannula. Proper adjustment of the anesthetic was secured before the records were taken.

The pressure of the cerebrospinal fluid was measured in a U-manometer connected with a needle inserted into the cisterna cerebellomedullaris. Brachial venous pressure and the urinary output in drops were recorded as before, and the carotid arterial pressures taken by means of the mercury manometer. Intracranial arterial pressure was not recorded because of the impossibility of securing animals of sufficient size, but the correspondence of carotid pressure with those obtained for the intracranial arterial pressures has been shown to be so close that it was felt that the pressure in the common carotid artery would give adequate information regarding the cranial arterial circulation.

The choice and proper employment of a technical procedure for the recording of the pressure in the dural venous sinuses were considered to be of the utmost importance for this study. Hill (7) made use of an approach through the occipital bone to the torcular herophili and this method has been employed with slight modifications by Dixon and Halliburton (3), by Frazier and Peet (6), and by Becht (1). The disadvantages of the technique are due to the fact that the torcular in both cats and dogs lies well embedded in the bony skull and in addition the likelihood of extensive clotting seems to affect the accuracy

of the readings. Becht has already commented upon the necessity of careful control of this clotting in the torcular. The wide divergences of the torcular pressures reported have indicated that the technical procedure is not ideal.

After study of the cranial topography, a method of obtaining the venous pressure in the superior sagittal sinus as it empties into the torcular herophili was worked out and found to be satisfactory for the particular problem. In this experimental procedure the superior sagittal sinus was exposed over the posterior one-third of its course by carefully removing the bone in a small sagittal groove by means of a rongeur. This exposure was quickly made without injury to the dural walls of the sinus and in the usual experiment the dark channel of the venous sinus was seen coursing in the bony opening. The pressure of the cerebrospinal fluid was then taken and the readings of the normal level were obtained. With the manometer for the fluid pressure in place, a shortened lumbar puncture needle (17G) was inserted posteriorly in the superior sagittal sinus to the torcular herophili. This procedure was customarily effected without visible change in the pressure of the cerebrospinal fluid; it was held essential that no change in pressure of the fluid should be brought about by the introduction of the needle for the recording of sinus pressure. The needle after insertion was then firmly held in place and the bony groove filled with bone wax to prevent bleeding. With the withdrawal of the stylet from the needle, blood came freely from the open end; connection was then quickly made to a calibrated manometer of 1 mm. bore, filled with a 4 per cent solution of sodium citrate. The insertion into the system of a three-way cock permitted washing out of the needle, so that the system was constantly free from blood clot and pulsations of from 2 to 4 mm. excursion were observed throughout. For dependable readings it was considered necessary that such pulsations should be constantly present and that at the end of the experiment blood should come freely from the end of the needle on disconnecting the manometer. The values of the 4 per cent citrate solution were transposed into terms of Ringer's solution so as to be directly comparable to the pressures of the cerebrospinal fluid and of the brachial vein.

The advantages of this method of recording the pressure of the superior sagittal sinus at the torcular herophili lie in the simplicity of the procedure, the obtaining of free pulsation in the manometer, and, most important of all, the fact that the influence of the introduction of the needle into the sinus upon the pressure of the cerebrospinal fluid can

be observed. The importance of this latter fact cannot be over-emphasized in the securing of dependable data for, as is well known, increase of cerebral venous pressure inevitably elevates the cerebrospinal fluid pressure. With care in insertion of the needle, it was found possible to carry out the procedure with no effect on the cerebrospinal fluid pressure in practically every animal; in some cases the initial position of the needle required adjustment before the pressure of the cerebrospinal fluid was restored to its normal level. All experiments in which the procedure affected the pressure of the cerebrospinal fluid (and this effect was always an increase in pressure due to venous congestion in the cranium) were discarded.

Control observations. Under constant experimental conditions, with previous regulation of the anesthetic, a number of control observations was carried out, with the manipulative procedures limited to those required for the attachment of the recording instruments. Such experiments were necessary for the establishment of standards upon which were to be based the interpretations of the alterations of pressure occasioned by the intravenous injection of solutions of various concentrations.

Chart 1 gives a graphic representation of the pressures recorded in such a control observation upon a dog. In this animal during a 2-hour interval the pressure of the cerebrospinal fluid varied between 152 and 161 mm., the fluctuations in pressure being slow and fairly uniform. Throughout the experimental period, the excursions of the cerebrospinal fluid in the manometer were wide (over 4 mm.) indicating that no obstruction to the communication between subarachnoid space and manometer existed. The carotid systolic pressure showed a gradual ascent throughout the 2-hour interval, a change in tension which was apparently without effect upon the other pressures. The brachial venous pressure, in the early minutes maintaining a level slightly above 100 mm. of Ringer's solution, rose about 20 mm. after the first 15 minutes of observation—a slow rise occupying about 20 minutes. From this slightly higher level a gradual fall in pressure occurred, so that at the end of the observation the brachial venous pressure was but slightly above the initial level. This change in brachial venous pressure also was without apparent effect upon the other pressures. Urinary output throughout the first 90 minutes of the observation was quite normal for an animal under ether; it was somewhat irregular, though within physiological limits. During the last half-hour of the record practically no urinary output was observed.

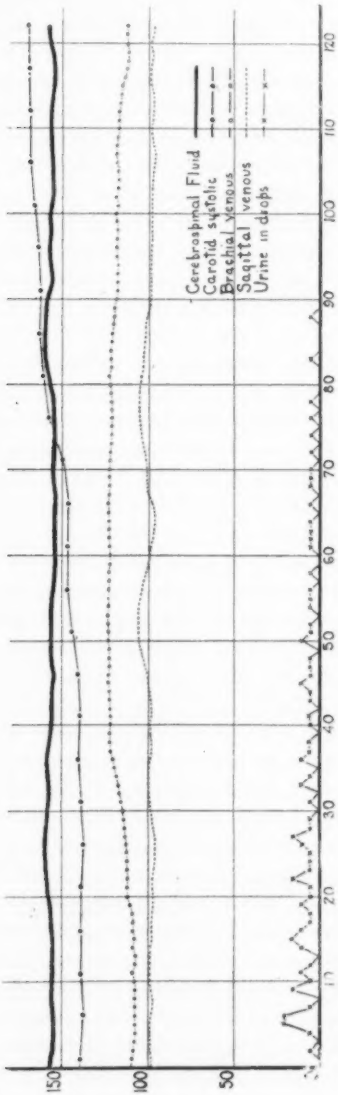

Chart 1. Experiment 113. Dog, weight 7450 grams. Ordinates represent millimeters of Ringer's solution or mercury (carotid pressure) and drops of urine (50 corresponding to 10 drops); abscissae represent time in minutes. Control observation for ether anesthesia.

Such a record, with the exception of the slowly rising pressure of the carotid, is quite similar to that given as chart 1 in the first paper of this series (10).

In addition to the pressures already commented upon, there is illustrated in chart 1 the record of the venous pressure of the superior sagittal sinus. This initially was recorded at 98 mm. of Ringer's solution; its greatest height during the 2-hour period was 105 mm. and lowest point 95 mm. The pressures therefore were remarkably constant and the fluctuations recorded were but very slow rises and falls. In this animal, the pulsations in the manometer connected with the sagittal sinus continued freely throughout; at the termination of the experiment blood flowed freely from the needle on disconnecting the manometer.

Apart from demonstrating the constancy of the experimental conditions under which this work was carried out, chart 1 is of interest in showing the relationship between cerebrospinal fluid and the sagittal venous pressure. The pressure of the cerebrospinal fluid throughout the 2-hour interval maintained a level of roughly 50 mm. above that of the superior sagittal sinus. This difference in levels was more marked in this particular animal than was usually the case, for in general we have found the cerebrospinal fluid pressure to be about 20 to 30 mm. higher than the sagittal pressure. Measurement of the two pressures in many animals in this series was convincing in demonstrating that the pressure of the cerebrospinal fluid practically always exceeds that of the superior sagittal sinus at the torcular herophili. With properly conducted experimental procedures, this general statement of pressure-differences has been found to be correct; with faulty recording instruments due to blood clots, leaks, etc., any inequalities in pressure may be found. Blood clots about the end of the sagittal needle caused many unreliable records, for when such clots occurred the sagittal pressure assumed levels of extraordinary magnitude while the pressure of the cerebrospinal fluid frequently remained unaffected. When the blood clots became extensive enough to cause congestion in the cerebral veins, the pressure of the cerebrospinal fluid was elevated.

The statement that the pressure of the cerebrospinal fluid is normally above that of the superior sagittal sinus is based on the almost invariable findings in these experiments; a single exception should however be reported. In this observation, a control in which the animal was maintained under ether without other procedure, the initial pressure of the cerebrospinal fluid was 107 mm., while that in the superior sagit-

tal sinus was 115 mm. Exclusion of technical errors convinced us that the values as determined were correct. The animal was maintained under constant experimental conditions; the cerebrospinal fluid pressure continued with slight fluctuation on its initial level while the sagittal venous pressure fell slowly, to become after 70 minutes below that of the cerebrospinal fluid. Throughout the remainder of the observation period of 4 hours, the cerebrospinal fluid pressure remained above that of the sagittal sinus, which gradually came to fluctuate about that of the brachial venous pressure. In all the other observations in which an initial reading of the cerebrospinal fluid above that of the superior sagittal sinus was recorded, technical errors were found to account for the discrepancy. It would seem logical to conclude, then, that under experimental conditions assuring constancy of observation, the pressure of the cerebrospinal fluid is above that of the superior sagittal sinus; the converse may, under certain conditions, be true but these conditions must be considered as exceptional.

This usual relationship of the cerebrospinal fluid pressure higher than that of the superior sagittal sinus is contrary to the view of Hill (7), of Dixon and Halliburton (3) and differs somewhat from the findings of Becht (1). Leonard Hill felt that the cerebrospinal fluid and torcular venous pressures must at all times be equal, while Dixon and Halliburton held that extreme variations between the two pressures might occur but that as a general law, the torcular venous pressure was higher than that of the cerebrospinal fluid. Becht concluded that either the torcular venous or the cerebrospinal fluid pressure might be higher than the other but that in general the venous pressure was the greater. These different views will be discussed later in this paper.

Ringer's solution. With the establishment of experimental conditions under which uniform records of the various pressures were obtained, the effects of relatively large intravenous injections of Ringer's solution were tested. The formula for the Ringer's solution was the same as that given in the initial paper in this series; the solution was injected at body temperature. Dogs were used exclusively for this phase of the investigation.

In chart 2 the record of such an experiment is illustrated. During the initial control period of 7 minutes, the pressure of the cerebrospinal fluid was constant at 129 to 130 mm., while that of the superior sagittal sinus was similarly constant at 107 to 108 mm. Brachial venous pressure was slightly higher than that of the superior sagittal sinus while

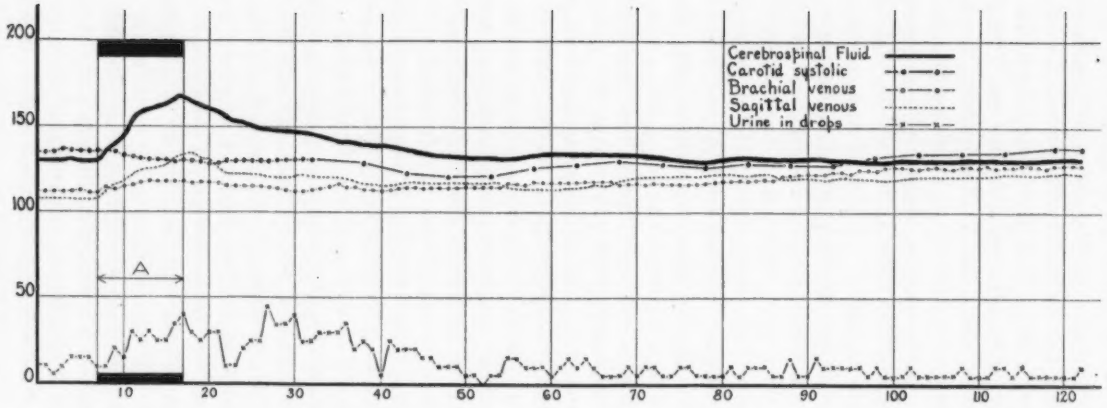

Chart 2. Experiment 112. Dog, weight 6750 grams. Ordinates represent millimeters of Ringer's solution or mercury (carotid pressure) and drops of urine (50 corresponding to 10 drops); abscissae represent time in minutes. During interval *A*, intravenous injection of 150 cc. of Ringer's solution.

the carotid systolic pressure hovered about 135 mm. Hg. Urinary output was normal. During the period *A*, an injection of 150 cc. of Ringer's solution was given intravenously at a rate of 15 cc. per minute. The resultant changes in pressure were quite similar to those of chart 2 of the first paper of this series (the doses corresponding roughly to the body weight) and to figure 3 given by Weed and McKibben (12). Both the cerebrospinal fluid and the sagittal venous pressures rose during the interval of injection, the former mounting from 130 to 167 mm. while the latter increased from 107 to 133 mm. This rise in cerebrospinal fluid pressure of 37 mm. may be compared to the increase of 26 mm. in the sagittal venous pressure. Brachial venous pressure during this period rose less than 10 mm., while the carotid systolic pressure fell slightly. Urinary output was increased during the injection.

Following this interval of administration of the Ringer's solution, the pressure of the cerebrospinal fluid fell gradually, regaining its former level in 30 minutes. This level was maintained with but minimal fluctuations throughout the rest of the 2-hour interval. The pressure in the superior sagittal sinus, which did not reach its height until 1 minute after cessation of the injection, fell somewhat more rapidly than the pressure of the cerebrospinal fluid for the first 5 minutes and then fluctuated both above and below the brachial venous pressure, on a level slightly increased over the initial readings. With the exception of the less marked increase during the injection-interval, the brachial venous pressure followed the same curve of reaction as did that of the superior sagittal sinus. In the initial period the systemic venous pressure was a few millimeters above the intracranial; during the last 80 minutes of the curve they were quite similar, at times one and at times the other being a few millimeters higher. The record of both pressures, altered only during the period of injection, is remarkably constant throughout. The carotid systolic pressure recovered but slightly in the minutes immediately following the injection and reached a somewhat lower level within 30 minutes afterward. From this point on the curve of carotid pressure with but minimal fluctuation was slightly upward. The urinary output was slightly increased for the first half-hour after the injection and then was continued at approximately the normal rate throughout the period of observation.

The pressures recorded in chart 2 are of interest in showing the correspondence of the intracranial and systemic venous pressures. While the cerebrospinal fluid and the two venous pressures were all

affected by the injection, disproportionate increases occurred. Of the three, the cerebrospinal fluid increased most markedly while the intracranial venous pressure showed a more striking increase than did the brachial venous pressure. This would indicate that the increase in the pressure of the cerebrospinal fluid had affected to a slight degree the intracranial venous pressure, and that in the rigid cranium this influence had resulted in a greater proportionate increase in venous pressure than was shown in the unrestricted brachial vein. With the fall in the pressure of the cerebrospinal fluid to its initial level, the intracranial venous pressure again became almost identical with the brachial venous pressure. This correspondence of the two venous pressures was maintained throughout.

The physiological reactions illustrated in chart 2 may be taken as quite typical of this series of experiments in which relatively large intravenous injections of Ringer's solution were given.

Hypotonic solutions. The augmenting effect of an intravenous injection of a relatively large quantity of a hypotonic solution (distilled water) upon the pressure of the cerebrospinal fluid has been well studied (Weed and McKibben (12), Cushing and Foley (2), Foley and Putnam (5), Ebaugh and Stevenson (4)). In the initial paper of this series, the general systemic vascular alterations following such injections were reported (10); similar effects were obtained in this group of experiments upon dogs to which large intravenous injections of distilled water were given. Typically, the rise of pressure of the cerebrospinal fluid was marked, abrupt and prolonged for some hours; occasionally the augmented pressure was maintained for only an hour or so and then fell to or below its initial level.

Chart 3 gives the tracings of the pressures of such an experiment upon the dog; the rise in the cerebrospinal fluid pressure was marked but was sustained only for an hour and then fell below its initial reading during the last few minutes of the record. It is given here, not only to show this relatively infrequent rapid recovery but to give greater evidence of the relationship of a rapidly changing cerebrospinal fluid pressure to the pressure in the superior sagittal sinus.

During the control period of 7 minutes in chart 3 the pressures were all constant: the cerebrospinal fluid at 122 to 123 mm., the sagittal venous at 59 to 62 mm., the brachial venous at 69 to 71 mm., and the carotid systolic at 90 to 93 mm. Hg. The intravenous injection of 150 cc. of distilled water was given during interval *A* at the rate of 15 cc. per minute—the same rate as used in the experiment repre-

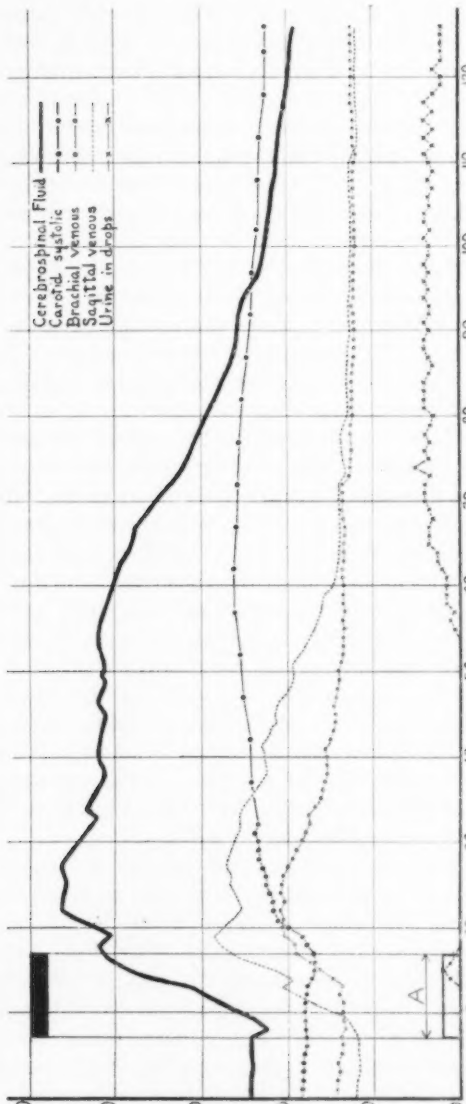

Chart 3. Experiment 109. Dog, weight 5400 grams. Ordinates represent millimeters of Ringer's solution or mercury (carotid pressure) and drops of urine (50 corresponding to 20 drops); abscissae represent time in minutes. During interval A, intravenous injection of 150 cc. of distilled water.

sented in chart 2. Both the cerebrospinal fluid and the sagittal venous pressures rose markedly during the period of introduction of fluid though the cerebrospinal fluid showed an initial drop during the first minute and the sagittal venous a slight rise. At the end of the injection-interval the pressure of the cerebrospinal fluid was 208 mm. (86 mm. above the initial). For 2 minutes after the injection was completed, the sagittal pressure continued to rise to 143 mm., while after a slight further increase of 2 mm. during the first minute following the injection, the cerebrospinal fluid pressure fell during the second minute 9 mm. Rebounding from this slight drop, the pressure of the cerebrospinal fluid mounted during the next 3 minutes reaching its maximum reading of 231 mm., while the sagittal pressure fell from 143 mm. to 127 mm. The pressure of the cerebrospinal fluid maintained this high peak for 6 minutes and then declined to a new level slightly above 200 mm., which was sustained for 40 minutes after the end of the injection. Meanwhile the sagittal venous pressure had risen slightly and then started an irregular, fluctuating descent which carried it at the end of the first hour of experimentation practically to the brachial venous pressure. With these pressure-changes taking place within the cranium, the brachial venous pressure had undergone alterations of somewhat the same character but of lesser magnitude than those of the superior sagittal sinus. During the injection-interval, no change in brachial venous pressure was recorded for the first 6 minutes; during the last 4 minutes the pressure mounted from 70 mm. to 96 mm. and then continued to rise for the next 5 minutes to 106 mm. From this high point it declined rather rapidly to its initial level of 70 mm. at the end of 30 minutes after the end of the injection. Carotid systolic pressure showed no change during the first half of the injection interval; then it fell slightly to rise rather slowly but regularly during the next 40 minutes.

At the end of the first hour of experimentation, then, cerebrospinal fluid pressure was still at 200 mm. while sagittal and brachial venous pressures had practically come together at about 70 mm. of Ringer's solution. During the second hour, cerebrospinal fluid pressure fell slowly but regularly to reach a level slightly below 100 mm. at the end of the 2 hour period. Sagittal and brachial venous pressures however maintained a level course slightly above 60 mm. during this hour, the former being for the most part higher than the latter, though in the last 15 minutes of the record the relations were reversed. Carotid systolic pressure continued throughout at a somewhat high level though

it declined slowly during this second hour. Output of urine began only at the beginning of this hour and was of moderate quantity throughout the period of experimentation.

The alterations in pressure illustrated in chart 3 are quite typical of the series. Worthy of note are the delayed and rather restrained reaction of the brachial venous pressure, the slight effect upon the carotid systolic pressure, and particularly the similarity, in initial reaction to the injection, of the cerebrospinal fluid and the sagittal venous pressures. The divorcing of the cerebrospinal fluid pressure from that of the superior sagittal sinus occurred quite soon; within 40 minutes after the completion of injection the sagittal and brachial venous pressure were returned practically to their original levels while the cerebrospinal fluid pressure was still 78 mm. above its initial reading. The rapid drop during the second hour of the pressure of the cerebrospinal fluid from 200 mm. to slightly less than 100 mm. was without effect upon either the sagittal or brachial venous pressure.

Such reactions as given in chart 3 were obtained in the experiments in this series; the data all show the close correspondence of the sagittal and brachial venous pressures. When the cerebrospinal fluid pressure was raised by the injection of distilled water, the pressure in the superior sagittal sinus increased to a greater degree than did that in the brachial vein; here again the mechanical effect of the increased pressure of the cerebrospinal fluid upon the cerebral veins may be the factor in causing this inequality in pressure between that of the vein enclosed within the cranium and that unconfined by bony walls.

Compilation of the reactions obtained in dogs from intravenous injections of distilled water has been made. The data when analyzed show that in dogs of an average weight of 6918 grams, as average intravenous injection of 137.5 cc. of distilled water was given—20.6 cc. per kilogram of body weight. The average elevation of the cerebrospinal fluid pressure was 95.5 mm.—4.5 mm. per cubic centimeter of water per kilogram.

Hypertonic solutions. Experiments similar to those already described were carried out with the intravenous injection of strongly hypertonic solutions (30 per cent sodium chloride). The profound reduction of the pressure of the cerebrospinal fluid caused by such intravenous injections has been repeatedly found by other workers in this field (Weed and McKibben (12), Cushing and Foley (2), Sachs and Belcher (8), Foley and Putnam (5), Ebaugh and Stevenson (4), Sachs and Malone (9)) but no one has thus far studied the effect of such injections

upon the intracranial and systemic venous pressures. In half of the animals the first effect of the intravenous injection of the concentrated solutions was found to be a fall in the pressure of the cerebrospinal fluid, the rise occurring immediately after; in other animals, at the onset of the injection, an immediate increase in the fluid pressure occurred. Rarely in cats and somewhat more frequently in dogs, the pressure of the cerebrospinal fluid was observed to decline immediately at the beginning of the injection and to show no tendency toward this initial rise.

On this account it is impossible to give in one chart all of the types of initial reaction and it has been decided therefore to reproduce here one chart which shows an initial drop in the pressure of the cerebrospinal fluid followed by a rebound. For in such a reaction the intracranial pressures must undergo their extreme alterations and the information given will be of greatest value in the discussion of the problem.

Thus in chart 4 there are reproduced the pressure changes effected in a dog by the intravenous injection of 30. cc. of a 30 per cent solution of sodium chloride. In the control period of 7 minutes the cerebrospinal fluid pressure was constantly at a higher level than that of the sagittal sinus, which in turn was but slightly higher than that of the brachial vein, the respective levels being approximately 125 mm., 80 mm. and 68 mm. These pressures were practically unvarying, the fluctuations as shown in the chart being minimal. Carotid pressure during this period was about 110 mm. Hg., and no urinary output was observed.

As shown in chart 4, the intravenous injection was given over a period of 6 minutes at the rate of 5 cc. of the 30 per cent solution of sodium chloride per minute. The cerebrospinal fluid and the carotid systolic pressures both fell during the first 2 minutes of this injection, the former falling from 125 to 80 mm., while the latter declined sharply from 110 to 74 mm. Hg. During these 2 minutes, however, the brachial venous pressure mounted from 69 to 86 mm. of Ringer's solution but the sagittal pressure rose during the first minute from 82 to 102 mm. and then during the second minute fell back to 88 mm. From these levels, 2 minutes after the beginning of the injection, all of the pressures increased but at vastly different rates. The carotid systolic pressure mounted slowly from 74 to 96 mm. Hg. at the end of the injection; the cerebrospinal fluid pressure rebounded to 132 mm.—a reading but slightly above the initial values in the control interval. Sagittal venous pressure, lagging somewhat as did the cerebrospinal fluid during the third minute of the injection-period, then rose rapidly to reach 165

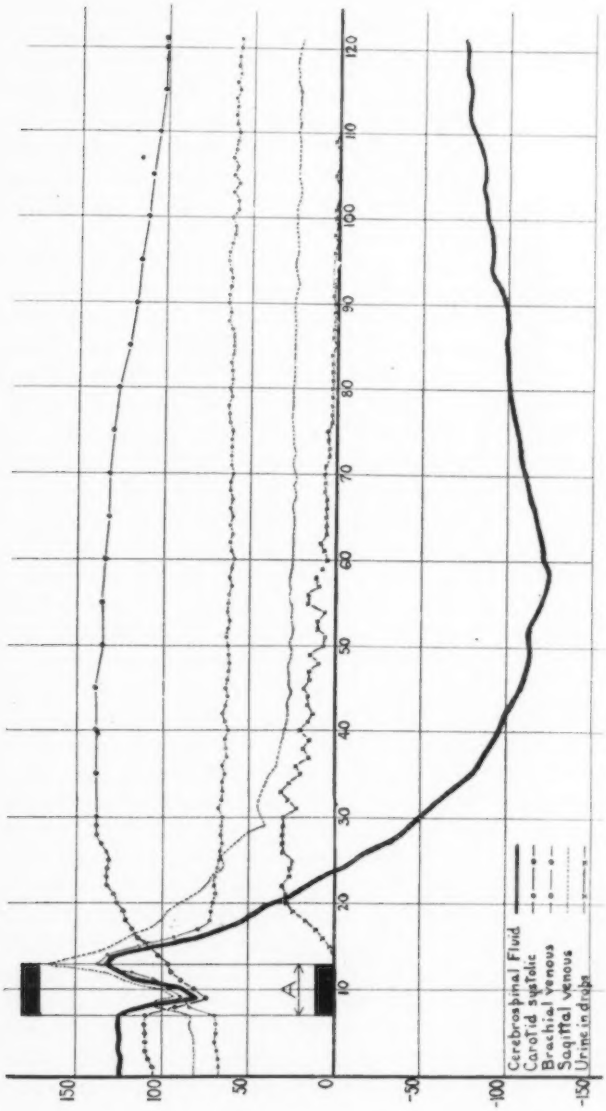

Chart 4. Experiment 97. Dog, weight 7450 grams. Ordinates represent millimeters of Ringer's solution or mercury (carotid pressure) and drops of urine (50 corresponding to 100 drops); abscissae represent time in minutes. During interval *A*, intravenous injection of 30 cc. of 30 per cent solution of sodium chloride.

mm. at the end of the injection. Brachial venous pressure showed increases in every minute of the injection-period though these increases were slight during the 2 minutes of lowered sagittal venous and cerebrospinal fluid pressures; at the end of the injection, brachial venous pressure was recorded as 136 mm. Thus, both the sagittal venous and the brachial venous pressures exceeded that of the cerebrospinal fluid at the end of the injection-interval though in the initial control interval both were considerably below the level of the cerebrospinal fluid pressure.

After the end of the injection the pressures all declined sharply with the exception of the carotid systolic which rose during the next 15 minutes to a new level at 138 mm. Hg., to be maintained for the next 50 minutes and then to decline slowly. The pressure of the cerebrospinal fluid fell with great rapidity, becoming negative in 10 minutes and reaching its maximum depression of minus 121 mm. in 45 minutes. From this time onward throughout the next hour of observation this pressure rose slowly to minus 72 mm. at the end of the record. Brachial venous pressure, paralleling the cerebrospinal fluid in its decline, assumed a new level slightly below its initial readings within 10 minutes after the end of the injection and continued throughout on this new level. Sagittal venous pressure, after reaching the height of 165 mm. at the end of the injection-interval, fell somewhat more slowly during the next 25 minutes when it reached a new low level of about 25 mm.–a level which it held with but slight fluctuation during the remainder of the record. Urinary output became marked following the injection and a polyuria of considerable degree existed for about 40 minutes. Coincidently with the tapering-off of this polyuria the slow rise in the pressure of the cerebrospinal fluid began.

Such reactions in the systemic and intracranial pressures have been found to be quite typical of the effect of intravenous injections of strongly hypertonic solutions. The carotid pressure, as already noted in the first paper in this series, usually fell during the period of introduction of the salt and then rather slowly rose to a new level above the initial value. The brachial venous pressure, as also noted before, rose markedly during the injection-interval, at times exceeding the pressure of the cerebrospinal fluid, and then declined to a new level, usually slightly below the initial readings. But most interesting in this particular phase of the general problem was the reaction of the intracranial venous pressure. In the experiment illustrated in chart 4, the sagittal venous pressure rose during the first minute of the experimental injection and

then fell, to rebound sharply to the highest level of the three pressures (sagittal venous, brachial venous and cerebrospinal fluid). In other experiments of this type an initial drop of the cerebrospinal fluid pressure did not occur; in these, with a marked rise in this latter pressure during the period of injection, the sagittal venous pressure rose constantly and at a faster rate, overtopping the cerebrospinal fluid pressure. This possibly indicated that the sharp fall in the sagittal venous pressure recorded in chart 4 was to be related directly to the fall in the pressure of the cerebrospinal fluid. This assumed relationship was further supported by the findings in those experiments in which an initial decline in the pressure of the cerebrospinal fluid followed the intravenous injection of the strongly hypertonic solution; in these the sagittal venous pressure mounted during the period of injection, occasionally at a slower rate than did the brachial venous pressure.

Following the completion of the injection, the sagittal venous pressure has been found to fall usually below the brachial venous pressure as illustrated in a typical experiment in chart 4. Here with a negative pressure within the cranium, the sagittal venous pressure still remained positive though at a low level, below the brachial venous pressure. Such findings suggested again an influence of the pressure of the cerebrospinal fluid upon the intracranial venous pressures. In one experiment of this type the intracranial venous pressure, as recorded in the superior sagittal sinus at the torcular herophili, became zero during the last part of the observation of 4 hours. But in no case, even with pressures of the cerebrospinal fluid as low as minus 185 mm. have negative pressures within the superior sagittal sinus been observed. In some experiments the sagittal venous pressures in the initial control-interval were considerably higher than that of the brachial vein but still below those of the cerebrospinal fluid. The final reaction in these cases was usually a reduction of the sagittal pressure to a level similar to that of the brachial vein.

Compilation of the reactions of the cerebrospinal fluid to such intravenous injections of 30 per cent solutions of sodium chloride have shown that in dogs of average weight of 7235 grams an average injection of 7.41 grams of sodium chloride was given intravenously—1.05 grams of sodium chloride per kilogram of body weight. The average fall in the pressure of the cerebrospinal fluid in these experiments was 238 mm., giving in every case negative pressures, or 234 mm. per gram of sodium chloride per kilogram of animal. This reaction was far more marked than that reported as the average for

cats in the first paper of this series; the difference may lie in the possible greater ease of reduction of the cerebrospinal fluid pressure in the dog but more likely is it that the age of the individual animals played a more important part. In our earlier studies we employed large cats which were usually old as judged by general appearance, teeth, etc.; the dogs used for this present series of observations, however, were all young, many of them sexually immature. Throughout this entire series of studies, the greater reactions have been noted to occur in the younger animals than in the old and the present compilation adds additional evidence to this general observation.

The present group of experiments demonstrates, then, the same general reactions, as before noted, effected by the intravenous injection of strongly hypertonic solutions, but with the striking difference that the sagittal venous pressure is lowered more markedly than is the brachial venous pressure.

Effect of abrupt change in cerebrospinal fluid pressure. With many findings indicating a close relationship between the reactions of the pressures in the superior sagittal sinus and in the superficial brachial vein, it was thought desirable to carry out an experiment of the type described in the second paper of this series (11). In this the cranium on one side was opened, the intact dura freed and the bony defect subsequently sealed. The pressures recorded were those of the cerebrospinal fluid, carotid artery, superior sagittal sinus and brachial vein. With the record of these pressures taken with an intact cranium, it was planned to open suddenly the cranial defect and observe alterations in pressure caused by this change.

Chart 5 presents in graphic form the record of an experiment on a dog performed under these conditions. In the control period of 9 minutes the cerebrospinal fluid pressure was high (about 175 mm.), the sagittal venous pressure slightly above 100 mm. and the brachial venous pressure 65 mm. During the injection-period *A*, the carotid systolic pressure fell slightly, rebounded and then dropped slightly again. Brachial venous pressure rose 52 mm.; cerebrospinal fluid pressure, after a slight initial drop, increased from 175 to 191 mm. while sagittal venous pressure mounted from 103 to 239 mm. These three pressures all fell following this first injection; after 15 minutes the sagittal venous pressure was only 15 to 20 mm. above the brachial venous pressure, as compared with a difference of 40 mm. before the injection. Cerebrospinal fluid pressure fell to 30 mm. following this injection; carotid pressure remained high. A second

intravenous injection of the hypertonic salt solution was then given rapidly during interval *B*; the arterial pressure dropped strongly while the sagittal venous pressure rose markedly. Brachial venous and cerebrospinal fluid pressures increased slightly and then declined following the injection. After 15 minutes the cerebrospinal fluid was strongly negative while the sagittal and brachial pressures had become

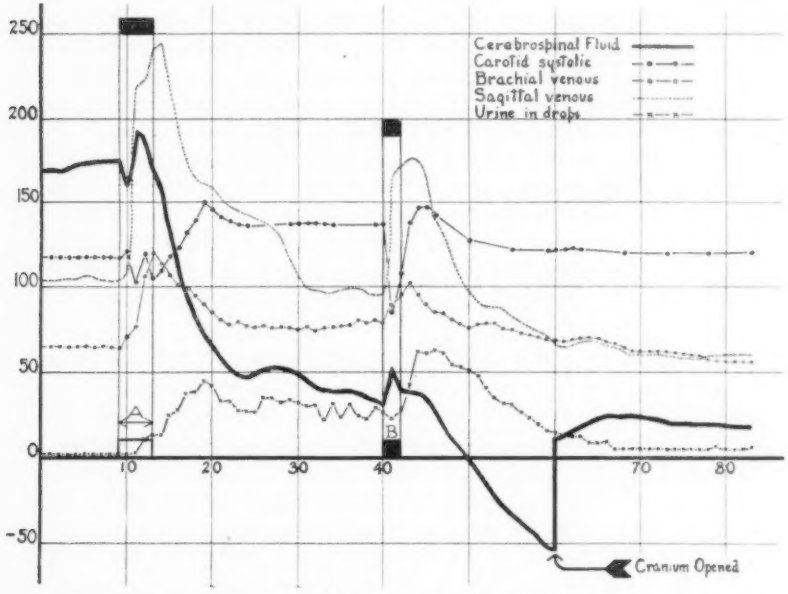

Chart 5. Experiment 110. Dog, weight 5850 grams. Ordinates represent millimeters of Ringer's solution or mercury (carotid pressure) and drops of urine (50 corresponding to 100 drops); abscissae represent time in minutes. During interval *A*, intravenous injection of 20 cc. of 36 per cent solution of sodium chloride; during interval *B*, intravenous injection of 11 cc. of same solution. Cranium opened and subsequently sealed.

practically identical at 70 mm. Eighteen minutes after the second injection the sealing slide was removed and the cranium opened; the pressure of the cerebrospinal fluid immediately rose from minus 52 mm. to plus 15 mm., while the carotid, sagittal and brachial venous pressures remained practically unaltered. During the next few minutes the dura was more completely freed from the skull,

and in consequence the cerebrospinal fluid rose to a somewhat higher level, but with the exception of a momentary increase of slight extent the sagittal and brachial pressures were unaffected, dropping slightly to a lower level. Carotid pressure, of which a continuous record was taken during the period of opening of the cranium, showed no change throughout.

The gradual approach of the sagittal venous pressure toward the brachial venous pressure and final maintenance of the same level with it has been noted in many experiments. The demonstration of the part played by the cranium in the formation of a rigid covering of the central nervous system was well shown by the change in pressure of the cerebrospinal fluid from negative to positive readings on removal of the sealing mechanism. But this abrupt mechanical change in the cerebrospinal fluid pressure was accompanied by only a momentary change in the sagittal venous pressure; any exact relationship of the two pressures or absolute influence of the cerebrospinal fluid pressure on the sagittal venous pressure was lacking.

DISCUSSION

In the foregoing sections of this paper there have been detailed the effects of the intravenous injection of solutions of various concentrations upon the cerebrospinal fluid, the sagittal and brachial venous pressures, the carotid systolic pressure and the urinary output. Data have been presented to show that under the conditions of experimentation, the pressure of the cerebrospinal fluid remains constant with only minimal fluctuation; brachial and sagittal venous pressures likewise show no marked or abrupt changes in tension. With the intravenous injection of Ringer's solution in large amounts, the short-enduring rise in the pressure of the cerebrospinal fluid is accompanied by a smaller rise in the sagittal venous pressure and by an even smaller increase in the brachial venous pressure. After the intravenous injection of a hypotonic solution (distilled water) the greatest rise occurs in the pressure of the cerebrospinal fluid, a lesser but still considerable increase in the sagittal venous pressure and a still smaller augmentation of the brachial venous pressure. Two phases occur in the reactions of these pressures to the intravenous injection of strongly hypertonic solutions: in the first, during the period of injection, striking increases are typically found in both brachial and sagittal venous pressures, the latter usually outstripping the former, while the cerebrospinal fluid pressure customarily rises after a frequently occurring initial fall. Following

this initial phase of reaction, all of these three pressures fall, the cerebrospinal fluid most markedly of all, frequently reaching negative values while the brachial venous pressure assumes a new level slightly below its initial value. Sagittal venous pressure is more markedly reduced than the brachial pressure and maintains a new level 20 to 40 mm. above zero though occasionally dropping to zero.

Analysis of these findings is essential for the proper study of the intracranial pressure relationships. It is important in the first place to consider the present observations in comparison with those of other workers, particularly in regard to the normal pressures. Leonard Hill (7) whose studies mark the beginning of the modern work on the physiology of the cerebrospinal fluid held strongly to the view that the pressure of the cerebrospinal fluid and that of the cerebral veins, as measured in the torcular herophili, must at all times be identical. Wegefarth's (14) experiments developed the idea of relative inequalities of cerebral venous and cerebrospinal fluid pressure, with the latter exceeding or being constantly reduced to the former. Wegefarth made an artificial opening between superior sagittal sinus and subarachnoid space in dogs; this opening persisted for at least 4 days. In only one case did slight bleeding from the sinus into the subarachnoid space occur and in this animal cerebrospinal fluid was removed at the time of the puncture of the sinus wall. If only a minimal amount of cerebrospinal fluid was withdrawn at the time of puncture of the sinus, no evidence of intra-meningeal bleeding was found. In such animals, however, withdrawal of the cerebrospinal fluid caused marked subdural and subarachnoid hemorrhage. The experiments indicated that the cerebrospinal fluid pressure was constantly above that of the superior sagittal sinus, and that blood did not pass from the sinus to the subarachnoid space unless cerebrospinal fluid was withdrawn in quantity sufficient to alter intracranial relationships. Dixon and Halliburton (3), however, presented data showing that the cerebrospinal fluid pressure was independent of the venous pressure in the torcular, and that of the two, the venous pressure was constantly the higher. They stated that (p.153) "the C.S. (cerebrospinal fluid) pressure is influenced passively to a small extent by changes in the arterial and venous pressures but such changes are insignificant compared with the independent changes in pressure which occur as the result of secretory activity." Shortly after this publication, Frazier and Peet (6) reported the effect of intravenous injection of various tissue extracts upon the secretion of cerebrospinal fluid. They stated

that the cerebrospinal fluid and torcular venous pressures were practically identical and while they determined the intracranial venous pressure in the torcular, they did not give any figures of the absolute values.

Becht (1) has recently published data pertinent to the present discussion. He determined that torcular venous and cerebrospinal fluid pressures were almost but not exactly equal and that no definite statement could be made as to which pressure was the higher. Under physiological conditions these pressures were observed to vary in the same degree and to some extent proportionately in nearly every case. Becht found also that while the pressure of the cerebrospinal fluid was altered by change in the torcular venous pressure, moderate increases or decreases in the cerebrospinal fluid pressure did not affect that in the torcular. He concluded that pressure of the cerebrospinal fluid was the result of at least two factors: influence of venous pressure and influence of arterial pressure, so that the pressure of the cerebrospinal fluid may in some animals be above that of the torcular.

The data obtained in this present investigation indicate that the pressure of the cerebrospinal fluid is not identical with either the sagittal or brachial venous pressure. In the control experiments and in the initial control periods of other observations, the cerebrospinal fluid pressure has been found to be from 5 to 50 mm. above that of the sagittal sinus; the pressure in the sagittal sinus was rarely markedly different from that of the brachial vein. In some animals the sagittal venous pressure was higher than the brachial venous pressure; in other animals it was below that of the brachial vein. In animals in which the cerebrospinal fluid pressure was initially high, the pressure in the superior sagittal sinus was usually, though not invariably, considerably higher than that of the brachial vein. This correspondence of the sagittal and brachial venous pressure seems important in the consideration of the whole phenomenon; the pressure of the cerebrospinal fluid, under normal conditions, was in our experience practically always higher than the sagittal and brachial venous pressures.

It must be acknowledged that the pressures of the superior sagittal sinus obtained in this investigation do not agree with those recorded by Dixon and Halliburton (3) and by Becht (1). The former reported typical values for the torcular venous pressure at 5 minutes intervals as follows: 350, 294, 280, 260, 280, 260, 300, 415, 225, 230, 220, 230, 260, 330, 240, 255 mm. of 10 per cent citrate solution, while the cerebrospinal fluid pressure varied in the same periods as follows: 95, 25,

30, 35, 55, 25, 80, 65, 65, 75, 70, 60, 55, 50, 80, 90 mm. of the same solution. Many of the pressures reported by Becht for the torcular herophili are more in accord with those obtained by us while other values are far above any observed in our method of pressure-determination; the following values taken at random from different tables given by Becht are fairly typical of his readings: 87, 125, 272, 75, 85, 208, 201, 108, 108, 601, 354, 425, 496, 356, 58, 150, 118, 84, 150, 142, 201, 108, 262, etc. Becht also reported measurements of the venous pressures taken from the axillary vein as it joined the jugular; the data obtained afforded him no basis for comparison with the pressures from the torcular herophili.

The observations forming the basis of this report have been quite convincing in regard to the relationships, existing under the experimental conditions, between the pressure of the cerebrospinal fluid and that in the superior sagittal sinus. The method employed for the determination of the latter pressure has been such that the venous pressure recorded was obtained in the superior sagittal sinus as it entered the torcular herophili; the procedure, permitting initial observation of the pressure of the cerebrospinal fluid, in no way disturbed this fluid pressure. This control of the experimental procedure is essential for the proper evaluation of the observations on pressure, for any method which causes abnormal congestion in the veins over the cerebral hemispheres immediately raises the pressure of the cerebrospinal fluid as well as that of the venous sinuses. The increase in the pressure of the cerebrospinal fluid is not, however, as Dixon and Halliburton (3) first demonstrated, proportionate to the increase in venous pressure. Hence, if the manipulative procedure is such as to obstruct the free outflow of blood from the dural sinus, high pressures in the torcular herophili will be obtained while the pressure of the cerebrospinal fluid, though somewhat elevated, will not be proportionately increased. The continuous observation of the cerebrospinal fluid pressure both before and during the connection of the manometer recording the intracranial venous pressure seems essential for the avoidance of this error in record of the venous and cerebrospinal fluid pressure. With these criteria established, it becomes possible to obtain readings of the pressures in the dural sinuses upon which reliance may be placed. Under these experimental conditions, the cerebrospinal fluid pressure has been found to be practically always, in control experiments and in the control periods of other observations, higher than the pressure of the superior longitudinal sinus. This statement of relative pressures is contrary

to that of Dixon and Halliburton and in accord only in part with that of Becht who feels that either of the pressures may be the higher. But this contention that the cerebrospinal fluid pressure is normally above that of the dural sinuses alone explains Wegefarth's (14) experiments, to which but little attention has been accorded. His demonstration that an opening can exist for at least 4 days between dural sinus and subarachnoid space without intrameningeal hemorrhage seems important support to the idea of a cerebrospinal fluid pressure constantly above that of the superior sagittal sinus. Wegefarth's ingenious experiment was free from the errors of recording instruments; the results were clear-cut and conclusive.

The disproportion between the pressures of the cerebrospinal fluid and the torcular herophili may also find explanation in the low pressure of the cerebrospinal fluid recorded by some workers. Pressures of the cerebrospinal fluid in adult animals much below 90 mm. have been found in a long series of observations to be quite abnormal and have almost always been found only in those animals in which the occipito-atlantoid ligament has been punctured more than once. In such cases, on failure of the initial puncture to yield cerebrospinal fluid, a second puncture, if successful, gives a low pressure of the cerebrospinal fluid. The explanation of this finding is apparently to be related to the escape of the cerebrospinal fluid through the initial puncture-hole of the ligament into the softer tissues of the neck. Occasionally the second puncture enters the ligament through the initial opening; in these animals replacement of fluid in the manometer suffices for the establishment of normal pressure-values. Consideration of these observations have made possible the exclusion of the data obtained from technically imperfect experiments; it would be of service to know the number of holes made into the subarachnoid space throughout the occipito-atlantoid ligament in the animals in which very low pressures were recorded.

The extremely high pressures in the torcular herophili reported by Dixon and Halliburton (3) (up to 415 mm. of 10 per cent citrate) and by Becht (1) (up to 600 mm. of NaCl solution of specific gravity of 1.088) have not been obtained by us when a different method of recording the pressure in the dural sinuses was employed. It may be argued that the method used in these experiments gave the pressure in the superior sagittal sinus and not in the torcular herophili, but this would be obviously an advantage as the venous pressure most desired for comparison would be that in the exposed veins over the

cerebral cortex and not in the dural sinuses where the elasticity of the containing wall is markedly less than that of the exposed vein. But by this method, immediately upon introduction of the needle into the sagittal sinus, there is sometimes obtained a very high initial determination of the venous pressure; in these cases there is always associated an increase in the pressure of the cerebrospinal fluid to a higher level, though below that of the venous sinus. Usually in such animals slight readjustment of the position of the needle will result in an extraordinary fall of the sinus pressure while the cerebrospinal fluid pressure returns quickly to its initial normal level. In rare instances readjustment of the needle has failed to modify the extraordinarily high venous sinus readings; on post-mortem examination a disproportion between the caliber of the needle and the sinus or an intravascular clotting due to extensive injury of the endothelium has been demonstrated. In the small sagittal sinus of cats, the difficulties occasioned by disproportionate size of the needle were almost constantly encountered; such animals gave enormously high pressures for the dural sinus with the cerebrospinal fluid pressure elevated but not to the same extent. With such experiences it becomes difficult to interpret the very high pressures in the superior sagittal sinus of normal animals as representing anything but experimental errors. Under the established conditions of observation, we have not found the intracranial venous pressure to be markedly above 100 mm.; in many of the observations it has been almost identical with that of the brachial vein. Many workers in this field have failed to record simultaneously the pressures of intracranial and systemic veins; Becht (1) considers the determination of systemic venous pressure to be without value. As affording controls for the determination of the true pressures in the dural sinuses, the brachial venous pressures have in this series of experiments been of greatest service and have permitted the elimination of unreliable data in many instances.

This correspondence of brachial and sagittal venous pressures seems a matter of great physiological interest. Under the alterations effected by the injections of solutions of various concentrations, this correspondence in pressures was amply demonstrated. Following intravenous injection of Ringer's solution, the sagittal venous pressure rose to a greater extent than did the brachial venous but within a few minutes both had returned to corresponding levels. With administration of distilled water, the sagittal venous pressure increased even to higher levels proportionately than did the brachial venous; the return to normal

levels of both pressures was somewhat slower. During the injection of strongly hypertonic solutions, however, the intracranial venous pressure exceeded in its marked rise the brachial venous pressure; but in the period following the injection the sagittal venous pressure fell below or assumed similar levels with the brachial venous pressure.

The explanation of these correspondences in venous pressures and their disproportionate changes does not seem impossible. At times the intracranial venous pressure, as measured in the superior sagittal sinus, lies at a higher level than does the brachial venous pressure; this correspondence in control observations is largely maintained. In other animals the pressures may be practically identical or the brachial venous slightly higher than the sagittal pressure. In both situations the pressures are determined by the general physiological conditions which affect venous pressures, plus the local conditions of external pressure. For the superficial brachial vein, there is but little of this modifying pressure and the rôle of the skin and underlying tissues must not be marked. But with the superior sagittal sinus, there exist all the modifying influences of the closed cranium (including the intracranial arterial supply) and the positive pressure of the cerebrospinal fluid. It is doubtful whether under normal conditions of pressure, the more or less resistant fibrous tissue walls of the large dural sinus are greatly affected by the positive pressure of the cerebrospinal fluid but certainly the unprotected cerebral veins must feel this positive pressure markedly. Consideration of these factors and of the possible differences in resistance to the return of this venous blood to the heart make it seem more than likely that the brachial and sagittal venous pressures under the conditions of experimentation should not be identical.

On similar bases, the disproportionate alterations in the two venous pressures following the different intravenous injections employed find explanation also. In those cases in which there follow elevations of the cerebrospinal fluid pressure, there occur increases in the sagittal venous pressure greater in magnitude than in the systemic brachial vein. If, however, there is in addition a general reaction of increased venous pressure (as evidenced in the brachial vein), the disproportion between intracranial and brachial venous pressure becomes greater. When, on the other hand, the cerebrospinal fluid pressure is markedly lowered (negative values) the intracranial venous pressure comes to lie below that of the brachial vein. Such alterations in the two venous pressures would seem to find their explanation in the passive effect of

the cerebrospinal fluid upon the intracranial venous pressure: this effect is not proportionately but only relatively shown. This apparent influence of the pressure of the cerebrospinal fluid upon the pressure of the dural sinuses is outspoken in the alterations of pressure brought about by the intravenous injection of solutions of various concentrations, but when a purely mechanical change in the cerebrospinal fluid pressure is achieved (as in experimental opening of the cranium in the presence of a negative cerebrospinal fluid pressure) the effects are not clear cut. It is difficult to believe that the explanation of mechanical effect does not apply in both cases; the magnitude of the change is probably the determining factor.

Leonard Hill (7) felt that both the cerebrospinal fluid and cerebral venous pressures must at all times be equal—a contention disproved by Dixon and Halliburton's (3) demonstration that the cerebrospinal fluid was independent in its pressure relationships from that of the cerebral veins. Becht (1) also concluded that the cerebrospinal fluid pressure was not identical with that of the torcular herophili and stated that when the two pressures are exactly identical and vary exactly in the same direction and to the same extent, communications exist between venous sinus and subarachnoid space. Our own experiences in this regard have led to similar conclusions; identical pressures in sagittal sinus and in cerebrospinal fluid with variations of the same degree and in the same direction have been found only in those animals in which technical errors have resulted in the artificial establishment of communications between subarachnoid space and venous sinus.

Becht (1) has attacked Dixon and Halliburton's (3) contention that alteration of the pressure of the cerebrospinal fluid changes in the same direction the pressure in the venous sinuses. It seems most probable that within definite physiological limits the view of Dixon and Halliburton is correct; the explanation ventured in the foregoing paragraphs for the disproportionate changes between sagittal and brachial venous pressures under the influence of intravenous injections of various concentrations involves acceptance of this view. As far as we are able to ascertain, no previous workers have observed such close correspondence between intracranial and brachial venous pressures and in consequence the general alterations in the venous system, apart from those occurring within the cranium, have not been extensively used as a basis for the interpretation of the intracranial changes. The alterations effected in the sagittal venous pressure by modifications in the pressure of the cerebrospinal fluid by injection of solutions of

various concentrations, are not proportionate to the changes in the fluid pressure; in general they are much less in extent, though always in the same direction, and may be determined by comparison with the general venous pressure. The converse of this proposition—that changes in the intracranial venous pressure affect the pressure of the cerebrospinal fluid in the same direction but not to the same extent—as first developed by Dixon and Halliburton and verified by Becht, is also demonstrated by the present findings following the injection of the different solutions. Here, again, the importance of comparison of the intracranial and systemic (as determined in the superficial brachial) venous alterations must be emphasized.

Another factor to which importance must be given in any discussion of intracranial relationships is the varying volume of the brain, as altered by the intravenous injection of solutions of different salt-content. As Weed and McKibben (13) reported, the injection of the strongly hypertonic solution decreases the size of the brain while the injection of the hypotonic solution causes an increase in its bulk. Such modification of the volume of the brain, under the influence of these solutions, inevitably must affect the pressures not only of the cerebrospinal fluid but of the cerebral veins; the arterial changes under these conditions are seemingly of little significance. At this time, while it is impossible to state with exactness whether the experimental alteration in the volume of the brain is a determining factor in the changes in the pressure of the cerebrospinal fluid, effected by these solutions of different concentrations, the combination of the two phenomena (alteration in brain bulk and change in the pressure of the cerebrospinal fluid) must be of utmost importance in the determination of intracranial tension. It is hazardous, without further data, to assign more than an indefinite function to the volume-alterations of the brain in this discussion of intracranial pressure-relationships, but increase in knowledge of the problem will in the future permit more concise statement. It seems essential that in the analysis of experimental work upon the cerebrospinal fluid, consideration be given to this possibility of change in the volume of the brain.

Consideration of all of the findings reported in this communication suggests that the intracranial arterial pressure is an important factor in maintaining the intracranial pressure conditions but its importance may be over-emphasized. Minor slow alterations of the cerebral arterial supply as determined by the carotid pressure seem to have no appreciable effect upon the pressure of either the cerebrospinal

fluid or the sagittal venous tension. Marked sudden alterations in the arterial supply to the structures within the cranial cavity, however, do affect the pressures of the cerebrospinal fluid and superior sagittal sinus—a conclusion held by both Dixon and Halliburton (3) and by Becht (1). This influence of arterial supply is, however, of apparently less physiological importance than is the venous pressure.

It becomes apparent that the pressure of the cerebrospinal fluid is, as Dixon and Halliburton first contended, independent of both intracranial arterial and venous tensions but is modified by both. Alterations in the pressure of the cerebrospinal fluid to a degree disproportionate to the changes in either intracranial venous or arterial pressures, may be brought about by the intravenous injection of solutions of various concentrations.

CONCLUSIONS

1. The alterations in the pressure of the cerebrospinal fluid, effected by the intravenous injection of solutions of various concentrations, are in large part independent of the alterations in the intracranial arterial and venous pressures.

2. The pressure of the cerebrospinal fluid, while dependent in part upon cerebral arterial pressure and in larger measure upon cerebral venous pressure, is independent of either.

3. The pressure of the cerebrospinal fluid, in the etherized animal under constant experimental conditions, is practically always higher than that of the superior sagittal sinus. This relationship holds during alterations in pressures effected by the intravenous injection of isotonic and hypotonic solutions; it is reversed after the intravenous injection of strongly hypertonic solutions.

4. Alterations in the intracranial venous pressure effect changes in the pressure of the cerebrospinal fluid, in the same direction but not to the same extent.

5. Within certain physiological limits, changes in pressure of the cerebrospinal fluid brought about by the intravenous injection of solutions of various concentrations, effect changes in the cerebral venous pressure as measured in the superior sagittal sinus.

6. A marked correspondence between venous pressures as determined in the superficial brachial vein and in the superior sagittal sinus seems demonstrated; the exact levels of the two pressures are modified by the local conditions of their situation.

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STUDIES IN THE PHYSIOLOGY OF VITAMINS

I. Vitamin-B and the Secretory Function of Glands[1]

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The question as to how vitamins function in the animal body is a very pertinent one. In answer to it several suggestions have been made. Uhlmann (1918), who made an elaborate study of the proprietary vitamin-B preparation Orypan, concluded that vitamin-B functions to stimulate glands and that its action is upon the nervous system like that of the drug pilocarpine, since he could abolish the alleged stimulating effect of Orypan by the injection of atropin. Verzar and Bögel (1920), on the other hand, were unable to demonstrate that extracts, which presumably contained accessory food factors, possessed any physiological properties when injected into the blood stream.

When experimenting with pigeons, Voegtlin and Myers (1919) noticed that the cure of a polyneuritic bird was accompanied by the evacuation from the bowel of a greenish colored fecal mass. Reasoning that the absence of vitamin-B resulted in a failure of the organism to elaborate the different secretions characteristic of the alimentary tract, these authors suggested that vitamin-B might be identical with the hormone *secretin*. They attempted to secure both vitamin-B from yeast and secretin from intestinal mucosa in a partially purified form, and compared their products as to their antineuritic and their secretin-like properties. The results, while not conclusive, were believed to point to the identity of these two substances.

[1] The data in this paper are taken from a dissertation presented by George R. Cowgill to the faculty of the Graduate School in Yale University, 1921, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

A preliminary account of some of the experiments was published by Cowgill, G. R.: Studies in the physiology of vitamins: Is water soluble vitamin identical with secretin? Proc. Soc. Exper. Biol. Med., 1921, xviii, 148–149.

The earliest suggestions and studies that were made to determine the exact rôle of vitamin-B naturally centered upon changes occurring in the nervous tissues. Funk (1912) compared the phosphorus and nitrogen content of the brains of normal and polyneuritic pigeons. There was a smaller amount of phosphorus compared to nitrogen in the case of the polyneuritic birds than in the case of the normal birds and this was interpreted to mean a loss of lipoids from the nervous system as a result of the disease. Schaumann (1911) put forward the hypothesis that the antineuritic vitamin functions as an activator stimulating the synthesis of phosphatides which are essential to the regeneration of nervous tissues.

Funk and von Schönborn (1914) asserted a relation to exist between the time required for polyneuritis to develop and the amount of carbohydrate contained in the diet. According to these authors, pigeons fed on a vitamin-free diet develop a hyperglycemia which is concomitant with a diminished glycogen content in the liver. The higher sugar content of the blood can be made to disappear and the content of liver glycogen to increase by supplying yeast vitamin. Funk believed, therefore, that vitamin-B functions in some way to aid in the synthesis of glycogen. This idea was tested later (Funk, 1919) by studying in normal and polyneuritic pigeons the action of substances which influence carbohydrate metabolism, namely, glucose, adrenalin, pituitrin, thyroid extract, parathyroid tissue and phlorhidzin. Except in the case of the thyroid extract, these substances did not in any way affect the onset of the hyperglycemia in birds fed a diet of polished rice.

The changes which the ductless glands undergo when the organism is placed upon a diet free from vitamin-B also have been investigated. Such studies have led to the idea that vitamin-B functions indirectly as a general metabolic stimulant. Funk and Douglas (1914) found that all glands atrophy and degenerate when the organism is placed on a vitamin-free diet. The thymus was found to involute under such conditions only to return to normal size when vitamin-B was fed. Dutcher (1919) and Novaro (1920) made similar observations concerning the testes, and McCarrison (1919) confirmed these results with other ductless glands. Such studies led to tests of thyroxin, desiccated thyroid, pilocarpine and tethelin to determine whether they possess antineuritic properties. According to Dutcher (1920), all of these substances produce definite relief and cessation of polyneuritic symptoms in pigeons but with a slower response than that which occurs when vitamin-B is fed. He believes that vitamins stimulate the ductless glands to activity in a manner which is yet to be discovered. Dutcher has suggested that the hormone supply of the body may be dependent upon the vitamin content of the food ingested.

Lumière (1920) fed pigeons on polished rice. Like many investigators, he observed that some of the birds failed to eat and therefore starved instead of developing polyneuritis. The starvation and its results were attributed to a failure of appetite due to a lack of digestive secretions, and Lumière believed that vitamins serve as stimuli to the glands of the digestive tract. Karr (1920) experimented on dogs and showed a relation to exist between appetite and the presence of

vitamin in the food, an observation which was confirmed by Cowgill (1921).

In view of these observations, experiments designed to test the effect of extracts containing vitamin-B on the secretory functions of different glands should be performed. Since Uhlmann, and Voegtlin and Myers[2] have alleged that vitamin-B stimulates glands upon what seems to the writers to be unconvincing experimental evidence, a reinvestigation of this problem with more rigid control procedures has been undertaken.

CRITIQUE OF PREVIOUS INVESTIGATIONS. Uhlmann (1918) presented several protocols of experiments designed to test the effect of the proprietary vitamin-B preparation Orypan upon the flow of pancreatic juice; they are very unconvincing. In many cases he failed to obtain a flow after injecting secretin; in some cases where the secretin solution proved to be a weak one, a series of injections of secretin was made at 2-minute intervals, and then followed within the short space of 5 minutes (after the secretin was producing a distinct and characteristic flow) by the vitamin-B injection, and this in turn followed after 10 minutes by another secretin injection. Bayliss and Starling (1902) showed that the effect of a potent secretin solution on the pancreas lasts from 12 to 15 minutes. Where repeated injections of secretin are made, the effect may be prolonged over a much longer period. This observation has been confirmed by other investigators, the writers included. Therefore when using secretin solutions as a control procedure in experiments such as Uhlmann and others performed, there should be a proper appreciation of this fact and the vitamin-containing product should not be injected until the effect of the secretin injection has very clearly passed away.

In view of the studies made by Pavlov (1901), Boldireff (1905) and Bayliss and Starling (1902), in which the rôle of acid in the duodenum has been demonstrated in the mechanism of pancreatic secretion, the activity of the alimentary tract in digesting and absorbing ingested food has been recognized as an important factor in determining at any time the normal rate of flow of pancreatic juice. In performing experiments designed to test the effect of a substance upon the rate of flow of this juice, three precautions seem to be suggested by the fact just stated: the animal should be starved for at least 24 hours; the

[2] During the preparation of this paper for publication, Anrep and Drummond (1921) published an account of experiments the results of which do not support the idea of Voegtlin and Myers that vitamin-B and secretin are identical.

pylorus should be occluded; and the flow of juice should be observed for a period long enough to give an adequate indication of its rate, without any special stimulus being applied.

In performing such experiments it is pertinent to ask: may not the vitamin-B promote the flow of pancreatic juice and bile when introduced first into the alimentary tract rather than directly into into the circulation?[3] It is perfectly evident that the introduction of the vitamin into the blood stream is an artificial and experimental procedure in no way comparable to what occurs normally when this dietary essential is ingested along with the other constituents of the diet. In answering this question experimentally, care must be taken that the preparation, which is introduced into the intestine, is not acid in reaction; the possibility of acid chyme being discharged from the intestine also must be eliminated by occlusion of the pylorus.

Bickel (1917) demonstrated that the hydrolysate of spinach produced by concentrated hydrochloric acid contained a substance which promoted the flow of gastric juice, and one of his students tested the effect of this hydrolysate upon the pancreas using an animal which had a Pavlov permanent pancreatic fistula. There was a marked increase in the flow of pancreatic juice; but, obviously, with a stimulating effect upon the gastric glands already demonstrated and with no precaution taken to prevent the pylorus from discharging acid juice, the proof that the effect of the hydrolysate upon the secretory activity of the pancreas is a specific one is inconclusive. The flow obtained may equally well have been due to secretin produced by the usual acid mechanism. This experiment serves to illustrate one of the common errors made in tests designed to demonstrate the occurrence of pancreatic succagogues.

In Voegtlin and Myers' (1919) experiments, which sought to prove the identity of vitamin-B and secretin, there was likewise a failure to occlude the pylorus.

There is a partial defense that might be offered for their procedure. The effect of secretin on the flow of pancreatic juice is characteristic producing a vigorous flow beginning about 1 minute after the secretin injection and lasting for from 12 to 15 minutes depending upon the amount of secretin injected, and it might be argued that this characteristic response to secretin injection can in no way

[3] As a matter of fact it has never been demonstrated, so far as the writers have been able to determine, that parenteral administration of vitamin-B to *mammals* brings about relief from polyneuritic symptoms; that such is the case is likely however, since it does occur with pigeons.

be confused with that which results from the discharge of acid chyme from the stomach. This is true provided the injected secretin preparation is a very potent one; where it is not so potent, one cannot be sure that the slight increase of flow is due only to the secretin injected. In most of the experiments reported in the literature, the increase in flow obtained subsequent to injections of different preparations was not very great.

Experiments designed to test the specific effect of vitamin-B preparations upon the flow of bile should be planned in such a manner that the flow obtained after injection of the preparation can be ascribed neither to the normal acid-secreting mechanism in the experimental animal nor to a stimulation of the musculature of the gall bladder wall. Occlusion of the pylorus and ligation of the cystic duct are control procedures essential to the proper performance of such experiments. Neither of these precautions was observed in the experiments performed by Voegtlin and Myers. Anrep and Drummond (1921) have called attention also to the fact that the flow of bile from the liver is more readily influenced by blood pressure changes than is the secretion from the other glands.

Experiments designed to test substances for their possible effect in stimulating the flow of saliva may be controlled *a*, by stimulating the chorda tympani nerve; *b*, by the injection of pilocarpine; *c*, by determining the effect of the substance upon blood pressure; and *d*, when a substance gives a positive effect, by a repetition of the test after section of the nerves to the gland.

Plan of the present investigation. In view of the fact that vitamin-B has not yet been isolated as a chemical individual, investigators of its physiological properties have been forced to use preparations containing the vitamin together with other substances. In this investigation, therefore, the tests that were made were not confined to any single product but to several in the hope that a parallelism might be established between a group of vitamin-containing preparations with respect to certain physiological properties.

The experiments herein described were undertaken to test experimentally the hypothesis that vitamin-B functions as a chemical entity which stimulates glands to secretory activity. Two important questions arise in this connection, concerning which different investigators have already expressed themselves, namely, 1, does vitamin-B possess properties similar to pilocarpine; and 2, is vitamin-B identical with the hormone secretin?

Preparations containing vitamin-B were made from wheat embryo, yeast, rice polishings and navy bean. Each product, while presumed

to contain the vitamin because of the method employed in its elaboration, was definitely proven to contain it by tests upon polyneuritic pigeons and dogs (Karr, 1920). Each product was also tested upon anesthetized dogs in which the rate of flow of secretion from the gland was noted before, during and after intravenous injection of the vitamin-B preparation. The vitamin-containing products were also injected into the stomach and intestine, and the effect of absorption of the vitamin from the alimentary tract upon the rate of flow of secretion determined. The pancreas, liver and salivary glands were studied. Dogs *1*, which had been fed an ordinary mixed diet, or *2*, which had lost their appetite after feeding for some time on a diet lacking vitamin-B, and *3*, polyneuritic dogs were used as test subjects in the experiments.

EXPERIMENTAL PART

Vitamin-preparations: Yeast. A sample of the Osborne-Wakeman (1919) concentrate of vitamin-B from brewery yeast was kindly furnished by Doctor Osborne for use in these experiments. This product was protein-free and contained the vitamin-B from approximately ten times its weight of dried brewery yeast. Fresh solutions were always used.

Wheat embryo, rice polishings and navy bean. Solutions containing vitamin-B were prepared from these substances as sources by the method described by McCollum and Simmonds (1918) but slightly modified so as to give an aqueous solution of the vitamin. The details of the procedures which were followed have already been described (Cowgill, 1921). The total solids contained in each solution thus prepared was determined.

Secretin. The lining of pig's duodenum and jejunum was scraped off and plunged into hot alcohol. After thorough hardening of the tissue, it was dried at room temperature, cut into fine pieces and preserved in a bottle. One and one-half grams of this dried product extracted over night by 100 cc. of 0.4 per cent hydrochloric acid furnished a potent secretin preparation. The total solids contained in the final neutral solution was determined. Solutions to be tested for their content of secretin were thus prepared from the intestines of polyneuritic dogs.

The few protocols which are presented in this paper are typical of many, all of which are contained in the dissertation. (See footnote 1.)

THE ACTION OF VITAMIN-B ON THE GLANDS OF ANIMALS FED A MIXED DIET: VITAMIN-B FROM WHEAT EMBRYO. *Tests of wheat embryo I.*

It contains vitamin-B. The presence of an abundance of vitamin-B in the preparation was shown by an experiment on a pigeon which, after being fed on polished rice exclusively for 16 days, developed a severe case of polyneuritis of the atrophic form. The bird was unable to stand, the neck was curled to the right, and the head was drawn under the body. Two cubic centimeters of Wheat Embryo I were injected into the left pectoral muscle. After 42 minutes the bird stood upon its legs but its neck was still twisted to the right and the head drawn under the body; at the end of an hour the bird was standing erect and was apparently normal.

It does not promote the flow of pancreatic juice. To test the possible action of this preparation upon the secretory activity of the pancreas two operations were performed upon dogs which had been fed the ordinary mixed diet consisting of dog meat and dog biscuit. In each case a pancreatic fistula was prepared, and ligation of the pylorus performed as a precautionary measure for the reasons already given. The following is a typical protocol.

PROTOCOL I. *Experiment 10.* Dog 8, male, weight 19 kilos, given injection of 0.085 gram of morphine sulphate. Ether followed by A-C-E mixture. Ligation of pylorus; cannulae in pancreatic duct and facial vein.

11:16.	Injected 5 cc. *secretin* solution; cannula promptly filled with secretion
11:30.	Injected 10 cc. *secretin*
11:30–11:35.	83 drops of pancreatic juice
11:35–11:40.	16 drops
11:40–11:45.	7 drops
11:45–11:50.	3 drops
11:50–11:55.	3 drops
11:56.	Injected 10 cc. *Wheat Embryo I* (= 10 grams)
11:56–12:02.	1 drop of pancreatic juice
12:02–12:07.	3 drops
12:07–12:12.	2 drops
12:12.	Injected 20 cc. *Wheat Embryo I* (= 20 grams)
12:12–12:18.	1 drop of pancreatic juice
12:18–12:23.	3 drops
12:23–12:28.	2 drops
12:28–12:31.	Injected 40 cc. *Wheat Embryo I* (= 40 grams)
12:28–12:33.	2 drops of pancreatic juice
12:33–12:38.	2 drops
12:38–12:43.	3 drops
12:43–12:50.	Injected 100 cc. *Wheat Embryo I* (= 100 grams)
12:43–12:48.	2 drops of pancreatic juice
12:48–12:53.	12* drops
12:53–12:58.	13* drops

12:58–1:03.	7* drops
1:03–1:08.	4* drops
1:08–1:13.	3 drops
1:13–1:18.	3 drops
1:20–1:26.	Injected 100 cc. *physiological saline* (0.9%)
1:20–1:25.	3 drops of pancreatic juice
1:25–1:30.	2 drops
1:30–1:35.	3 drops
1:35–1:40.	3 drops
1:55.	*Removed ligature from pylorus*
1:55–2:00.	3 drops of pancreatic juice
2:00–2:05.	2 drops
2:05–2:10.	2 drops
2:10–2:15.	2 drops
2:15–2:20.	1 drop
2:20–2:22.	Injected 50 cc. *Wheat Embryo I* (= 50 grams)
2:20–2:25.	3 drops of pancreatic juice
2:25–2:30.	3 drops
2:30–2:35.	3 drops
2:35–2:40.	2 drops
2:40–2:45.	2 drops
2:45.	Injected 5 cc. *secretin* solution
2:45–2:50.	33 drops of pancreatic juice
2:50–2:55.	8 drops
2:55–3:00.	3 drops

Injections to test potency of secretin solution

3:00.	Injected 1 cc. *secretin* solution
3:00–3:05.	4 drops of pancreatic juice
3:05–3:10.	2 drops
3:10–3:15.	2 drops
3:16.	Injected 2 cc. *secretin* solution
3:16–3:20.	12 drops of pancreatic juice
3:20–3:25.	3 drops
3:25–3:30.	2 drops

1 cc. secretin solution contained 0.004 gram total solids
1 cc. Wheat Embryo I contained 0.083 gram total solids

* Increased flow after injection of large dose of wheat embryo vitamin-B not confirmed by subsequent experiment.

It will be noticed in the experiment just described that a secretin-like action was obtained when the equivalent of 100 grams of wheat embryo was injected. An attempt was made to confirm this exceptional finding, but without success. The fact, however, that the other wheat embryo preparation which was used in this additional experiment,

had an abundance of vitamin-B—shown by tests upon polyneuritic pigeons and dogs—and yet did not have a secretin-like action when injected in large doses, indicates that the vitamin-B was not the agent responsible for the secretin-like action in the single trial cited. This fact also emphasizes the great importance of testing a variety of preparations all of which have the one factor in common, namely, a large content of vitamin-B.

In considering the experiment just described it surely cannot be claimed that too small doses of the vitamin were employed. Data presented elsewhere (Cowgill, 1921) show *a*, that the equivalent of 20 grams of ether-extracted wheat embryo introduced by sound into the stomach was sufficient to restore the appetite to a dog for 10 days, after the appetite had been lost due to feeding upon a diet lacking vitamin-B; and *b*, that the equivalent of 30 grams of the same material was sufficient to produce a distinct therapeutic effect in the case of the same dog when it developed severe symptoms resembling polyneuritis.

It does not promote the flow of saliva. Protocol IV gives the details of an experiment in which cannulae were placed in the ducts from the submaxillary and sublingual glands and the equivalent of 20 grams of ether-extracted wheat embryo was injected intravenously. As the protocol clearly shows, no salivary secretion was obtained.

SUMMARY: *Preparations of vitamin-B from wheat embryo,* although able to cure polyneuritic pigeons, to restore the appetite of a dog which had been fed a diet lacking the vitamin, and to produce a distinct therapeutic effect on a polyneuritic dog, *were without noticeable effect upon the secretory activity of the pancreas or the salivary glands of a normal dog.*

VITAMIN-B FROM YEAST. *Test of Osborne-Wakeman yeast fraction. It contains vitamin-B.* No experiments designed to test this preparation for its content of vitamin-B were made in connection with the present investigation, inasmuch as it had been tested many times by Osborne and Mendel and used as a source of vitamin-B in dietaries employed by them.

It does not promote the flow of pancreatic juice. An illustrative protocol follows.

PROTOCOL II. *Experiment 13.* Dog 6, male, weight 10.8 kilos, given 0.045 gram of morphine sulphate subcutaneously. Ether followed by A-C-E mixture. Ligature around the pylorus; cannulae in pancreatic duct and facial vein.

11:12.	Injected 10 cc. *secretin* solution
11:12–11:20.	42 drops of pancreatic juice
11:20–11:25.	9 drops
11:25–11:30.	2 drops
11:30–11:35.	2 drops
11:35–11:40.	3 drops
11:40.	Injected solution of 0.10 gram *Osborne-Wakeman concentrate* (= approx. 1 gram dried yeast)
11:40–11:55.	1 drop of pancreatic juice
11:56–11:59.	Injected 0.20 gram *yeast concentrate* (= approx. 2 grams dried yeast)
11:56–12:11.	1 drop of pancreatic juice
12:11–12:14.	Injected 0.30 gram *yeast concentrate* (= approx. 3 grams dried yeast)
12:11–12:26.	1 drop of pancreatic juice
12:26.	Injected 10 cc. *secretin* solution
12:26–12:29.	23 drops of pancreatic juice
12:29–12:36.	16 drops
12:36–12:41.	1 drop
12:41–12:46.	2 drops
12:46–12:51.	1 drop

1 cc. solution of O-W yeast concentrate contained 0.010 gram solids.
1 cc. secretin solution contained 0.004 gram solids.

In this experiment doses of the Osborne-Wakeman yeast fraction, which were equivalent to 1, 2 and 3 grams of dried yeast, were employed. Karr (1920) showed that 1½ gram of dried yeast was sufficient to restore appetite to dogs which had lost the desire to eat after being fed a diet lacking vitamin-B. He was even able in some cases to relieve polyneuritic symptoms in dogs by the administration of 3 grams of dried brewery yeast. We may conclude therefore that in the above experiment doses of vitamin-B of a magnitude sufficient to have a distinct effect upon dogs were used without having any effect upon the flow of pancreatic juice. Other experiments similar in nature might be cited, all of which likewise yielded a negative result. An attempt to influence the flow of pancreatic juice by introduction of the Osborne-Wakeman yeast fraction into the stomach and intestine was made and here again a negative result was obtained.

It does not promote the flow of saliva. The equivalent of 5 grams of dried yeast in the form of the Osborne-Wakeman yeast fraction was injected into a dog in which salivary fistulae had been made. Although the period of observation of salivary flow extended over a period of 1½ hours and three different vitamin preparations were injected, the only flow obtained was one drop from the sublingual gland, no flow whatever arising from the submaxillary gland. Stimulation of the

chorda tympani nerve both at the beginning and at the end of this experiment resulted in a flow from both glands thus proving that the mechanism was ready to function when given an appropriate stimulus. The vitamin-B preparations which were injected failed to supply this stimulus.

SUMMARY: The Osborne-Wakeman fraction of vitamin-B from yeast, although able to restore well-being to white rats which have been fed on a diet lacking this vitamin, is without any noticeable effect upon the secretory activity of the pancreas and the salivary glands, whether it be introduced intravenously or by way of the alimentary tract.

VITAMIN-B FROM RICE POLISHINGS: *Tests of Preparation I. It contains vitamin-B.* This preparation was able to bring about a complete relief from polyneuritic symptoms in a dog. A more detailed description of this animal is given elsewhere (Cowgill, 1921, chart 2 and protocol of dog 22).

It does not promote the flow of pancreatic juice or bile. The following experiment was performed.

PROTOCOL III. *Experiment 15.* Dog 28, female, weighing 7.2 kilos, was given 1.75 gram chloretone in saturated aqueous solution by stomach sound. Pylorus and cystic duct were ligated; cannulae placed in pancreatic duct and common bile duct. Injections were made into the facial vein.

Observation of normal rate of flow.

11:23–11:28.	0 drop pancreatic juice,	11 drops of bile
11:28–11:33.	1 drop pancreatic juice,	5 drops
11:33–11:38.	1 drop pancreatic juice,	? drops
11:51.	*Injected* 10 cc. *secretin* solution *intravenously*	
11:51–11:54.	14 drops pancreatic juice,	4 drops of bile
11:54–11:59.	56 drops pancreatic juice,	5 drops
11:59–12:04.	51 drops pancreatic juice,	15 drops
12:04–12:09.	11 drops pancreatic juice,	14 drops
12:09–12:14.	3 drops pancreatic juice,	13 drops
12:14–12:19.	1 drop pancreatic juice,	13 drops
12:19–12:24.	1 drop pancreatic juice,	13 drops
12:24–12:29.	1 drop pancreatic juice,	13 drops
12:29–12:34.	2 drops pancreatic juice,	11 drops
12:34–12:39.	1 drop pancreatic juice,	14 drops
12:39–12:40.	*Introduced* into *stomach* 50 cc. *Rice Polishings I* (= 100 grams)	
12:39–12:44.	0 drop pancreatic juice,	15 drops of bile
12:44–12:49.	0 drop pancreatic juice,	13 drops
12:49–12:54.	0 drop pancreatic juice,	12 drops
12:54–12:59.	1 drop (at 12:55),	9 drops
12:59–1:04.	0 drop,	9 drops
1:04–1:09.	1 drop (at 1:07),	8 drops
1:09–1:14.	0 drop,	8 drops

1:14–1:19.	1 drop (at 1:18),	8 drops
1:19–1:24.	0 drop,	7 drops
1:24–1:29.	0 drop,	10 drops
1:29–1:34.	1 drop (at 1:32),	8 drops
1:34–1:39.	1 drop (at 1:38),	8 drops
1:39.	*Introduced* into *intestine* 50 cc. *Rice Polishings I* (= 100 grams)	
1:39–1:44.	0 drop pancreatic juice,	5 drops of bile
1:44–1:49.	0 drop,	0 drop (obstruction ?)
1:49–1:54.	1 drop (at 1:52),	0 drop (obstruction ?)
1:54–1:59.	1 drop (at 1:55),	7 drops
1:59–2:04.	0 drop,	9 drops
2:04–2:09.	0 drop,	6 drops
2:09–2:14.	1 drop at (2:10),	8 drops
2:14–2:19.	0 drop,	8 drops
2:19–2:24.	0 drop,	7 drops
2:24–2:29.	0 drop,	5 drops
2:29–2:34.	1 drop (at 2:30),	8 drops
2:34–2:39.	0 drop,	7 drops
2:39.	*Removed ligature* from pylorus	
2:39–2:44.	1 drop (at 2:44) pancreatic juice,	15 drops of bile
2:44–2:49.	0 drop,	10 drops
2:49–2:54.	0 drop,	8 drops
2:54–2:59.	1 drop (at 2:58),	11 drops
2:59–3:04.	0 drop,	11 drops
3:04–3:09.	1 drop (at 3:08),	13 drops
3:09–3:14.	0 drop,	10 drops
3:14–3:19.	1 drop (at 3:15),	11 drops
3:19–3:24.	1 drop (at 3:21),	12 drops
3:24–3:29.	1 drop (at 3:27),	13 drops
3:29–3:34.	1 drop (at 3:34),	12 drops
3:34–3:39.	0 drop,	10 drops
3:39–3:44.	0 drop,	8 drops
3:39–3:44.	*Injected intravenously* 30 cc. *Rice Polishings I* (= 60 grams)	
3:44–3:49.	2 drops pancreatic juice,	4 drops of bile
3:49–3:54.	2 drops,	4 drops
3:54–3:59.	3 drops,	13 drops
3:59–4:04.	3 drops,	21 drops
4:04–4:09.	1 drop,	21 drops
4:09–4:14.	2 drops,	14 drops
4:14–4:19.	2 drops,	14 drops
4:19–4:24.	2 drops,	14 drops
4:24–4:29.	2 drops,	14 drops
4:29–4:34.	2 drops,	14 drops
4:34–4:39.	2 drops.	15 drops
4:40–4:43.	Injected 10 cc. *secretin* solution	
4:39–4:44.	72 drops pancreatic juice,	28 drops of bile
4:44–4:49.	54 drops,	14 drops

4:49–4:54.	14 drops,	15 drops
4:54–4:59.	5 drops,	13 drops
4:59–5:04.	2 drops,	14 drops
5:04–5:09.	2 drops,	15 drops

Experiment concluded. Ligature of cystic duct verified.

1 cc. secretin solution contained 0.004 gram total solids.
1 cc. Rice Polishings I contained 0.128 gram total solids.

The important thing to note in the preceding protocol is that intravenous injection of the rice polish preparation after the ligature around the pylorus had been *removed* resulted in an increased flow of both pancreatic juice and bile. The period of observation lasted 55 minutes and the character of the flow indicated that it was due not to a true secretin contained in the rice polish preparation but rather to the discharge of acid chyme from the stomach and the production of a small amount of secretin in the intestine of the animal. It will be noticed that the flow obtained amounted to only 2 drops of pancreatic juice every 5 minutes—not a great flow; it was, however, a distinct increase over the rate of flow which had obtained during the previous 3 hours. Reference to many protocols which might be cited shows that in many of the animals experimented upon, the normal rate of flow of pancreatic juice during a long period of observation after ligation of the pylorus was about 2 drops every 5 minutes. The results obtained in the above experiment find a very simple explanation if we consider that the rice polish preparation, when injected intravenously, promotes a discharge of acid chyme from the stomach and the consequent production of secretin in the small intestine.

This experiment shows how important it is, when studying the action of reputed secretagogues or secretins and substances of such a character upon the pancreas, to distinguish carefully between a true secretin which acts directly upon the gland through the medium of the blood stream and an indirect effect attributable to the discharge of stomach contents through the pylorus.

It does not promote the flow of saliva. Rice Polish I and Wheat Embryo III were tested on a dog having a temporary salivary fistula. The protocol follows.

PROTOCOL IV. *Experiment 23.* Dog 30, male, weight 9.9 kilos, given 2 grams of chloretone by stomach sound. Cannulae in submaxillary and sublingual ducts; cannula for injections in the facial vein; blood pressure from femoral artery.

Electrical stimulation of chorda tympani: vigorous flow of saliva.

11:14–11:21. Injected 11 cc. *Rice Polish I* (= 22 grams)
11:14–11:37. No flow of saliva
11:37–11:47. Injected 11 cc. *Wheat Embryo III* (= 20 grams)
11:37–12:22. No flow of saliva
12:22. Injected 20 mgm. *pilocarpine hydrochloride*
12:22–1:30. 154 drops from submaxillan, 13 drops sublingual

SUMMARY: A preparation of vitamin-B made from rice polishings, which relieved the symptoms of polyneuritis in a dog, showed no effect on the rate of flow of pancreatic juice, bile and saliva.

VITAMIN-B FROM NAVY BEAN: *Tests of Preparations I and II. I contains vitamin-B.* Pigeon 8 developed polyneuritic symptoms after feeding 26 days on a diet of polished rice. Four cubic centimeters of Navy Bean I were injected intramuscularly with complete relief of the symptoms.

II contains vitamin-B. The effect of this preparation on the food intake of a dog, which had lost its appetite after feeding on a diet lacking vitamin-B, has already been described (Cowgill, 1921, chart 3).

Navy Bean II does not promote the flow of pancreatic juice and bile. This preparation was tested upon an animal in which temporary pancreatic and biliary fistulas had been made. As the following condensed protocol clearly shows, the results were negative.

PROTOCOL V. *Experiment 16.* Dog 32, female, weight 11.8 kilos, given 2.15 grams chloretone by stomach sound. Ligatures around pylorus and cystic duct; cannulae in pancreatic duct and common bile duct; injection cannula in femoral vein.

10:20.	Injected 5 cc. *secretin* solution to fill cannula	
10:20–10:25.	9 drops of bile,	8 drops of pancreatic juice
10:25–10:30.	16 drops,	9 drops
10:30–10:35.	17 drops,	1 drop
10:35–10:40.	13 drops,	1 drop
10:40–10:45.	13 drops,	0 drop
10:45–10:47.	*Injected* 10 cc. *Navy Bean II* (= 20 grams). Dyspnea	
10:45–10:50.	11 drops of bile,	0 drop of pancreatic juice
10:50–10:55.	5 drops,	1 drop (at 10:54)
10:55–11:00.	4 drops,	0 drop
11:00–11:05.	5 drops,	1 drop (at 11:00)
11:05.	*Introduced* 50 cc. *Navy Bean II into stomach* (= 100 grams)	
11:05–12:00.	16 drops of bile every 5 minutes,	10 drops of pancreatic juice or approx. 1 drop every 5 minutes
12:00.	*Introduced* 50 cc. *Navy Bean II into intestine* (= 100 grams)	
12:00–1:00.	15 drops of bile every 5 minutes,	8 drops of pancreatic juice or approx. 1 drop every 8 minutes
1:00.	*Removed ligature* from around the pylorus	

1:00–2:00.	13 drops of bile, every 5 minutes,	4 drops of pancreatic juice or approx. 1 drop every 15 minutes
2:00.	Injected *intravenously* 10 cc. *Navy Bean II* (= 20 grams)	
2:00–2:30.	14 drops of bile,	2 drops of pancreatic juice
2:00–2:30.	14 drops of bile every 5 minutes,	2 drops of pancreatic juice
2:40–2:44.	Injected 15 cc. *secretin* solution	
2:40–2:45.	20 drops of pancreatic juice (beginning at 2:42)	
2:45–2:50.	12 drops	
2:50–2:55.	1 drop	
2:55–3:00.	1 drop	

Experiment concluded. Ligature around cystic duct verified.

1 cc. Navy Bean II contained 0.040 gram total solids.
1 cc. secretin solution contained 0.004 gram total solids.

Navy Bean I does not promote the flow of saliva. In two different experiments Navy Bean I was injected into the vein of a dog having a salivary fistula but with no effect whatever upon the flow of saliva although the secretion was readily obtained either by electrical stimulation of the chorda tympani nerve or by the injection of a small dose of pilocarpine.

SUMMARY: Preparations of vitamin-B from navy bean, which cured polyneuritis in pigeons and restored appetite to a dog that had been fed a diet free from this dietary essential, had no noticeable influence on the flow of pancreatic juice, bile or saliva.

THE ACTION OF VITAMIN-B ON THE GLANDS OF ANIMALS FED A DIET LACKING THIS DIETARY ESSENTIAL: *The flow of pancreatic juice in a dog which has lost its appetite. Vitamin-B does not influence the flow of pancreatic juice in such an animal.* Dog 16 was fed a diet lacking vitamin-B and on the 25th day absolutely refused the food offered. A chart, which is published elsewhere (Cowgill, 1921, chart 1) shows the food intake in this animal as it was influenced by supplying vitamin-B from wheat embryo. After a second refusal of the food offered lasting for 5 consecutive days, this animal was operated upon and a whole series of vitamin-B preparations tested for their possible influence upon the flow of pancreatic juice. The protocol follows.

PROTOCOL VI. *Experiment 21.* Dog 16, male, has refused the vitamin-free food for 5 consecutive days. Weight 7.8 kilos; given 1.8 gram chloretone by stomach sound; ligature around pylorus; injections into facial vein.

4:48–4:50.	Injected 15 cc. *Rice Polish I* (= 30 grams)
4:48–4:54.	2 drops of pancreatic juice
4:54–4:59.	1 drop

4:59–5:03. 1 drop
5:03–5:15. 5 drops
5:16–5:23. Injected 50 cc. *Navy Bean I* (= 100 grams)
5:16–5:31. 5 drops of pancreatic juice
5:33–5:38. Injected 25 cc. *Wheat Embryo II* (= 25 grams)
5:31–5:38. 3 drops of pancreatic juice
5:38–5:43. 2 drops
5:43–5:48. 2 drops
5:49–5:55. Injected 50 cc. of solution containing 5 *yeast vitamin* (Harris) tablets
5:48–5:54. 2 drops of pancreatic juice
5:54–5:59. 2 drops
6:00–6:02. Injected 25 cc. *tomato juice* neutralized 25 days before
6:00–6:05. 2 drops of pancreatic juice.
6:05–6:10. 1 drop
6:10–6:17. Injected equivalent of 5 grams of *Vegex*, a commercial extract of yeast. Dyspnea
6:10–6:15. 1 drop of pancreatic juice
6:15–6:20. 0 drop
6:20–6:25. 1 drop
6:25. Injected 10 cc. of *secretin* solution
6:25–6:30. 25 drops of pancreatic juice
6:30–6:35. 20 drops
6:35–6:40. 11 drops
6:40–6:45. 2 drops

Normal rate of flow estimated at 2 or 3 drops every five minutes.

1 cc. Rice Polish I contained 0.049 gram of solids.
1 cc. Navy Bean I contained 0.029 gram of solids.
1 cc. Wheat Embryo II contained 0.121 gram of solids.
1 cc. Harris yeast solution contained 0.039 gram of solids.
1 cc. Tomato preparation contained 0.049 gram of solids.
1 cc. Vegex preparation contained 0.064 gram of solids.
1 cc. Secretin solution contained 0.004 gram of solids.

Preparations containing vitamin-B were injected. Rice Polish I was shown to contain vitamin-B by a test on a pigeon which was in an advanced stage of polyneuritis of the spastic form. The bird was helpless. One hour after the intramuscular injection of 2 cc. of Rice Polish I there was complete relief from all symptoms. The tomato preparation brought about complete relief from all polyneuritic symptoms in the case of a dog. (See Cowgill, 1921, dog 25.) The proofs that Navy Bean I and Wheat Embryo II contained vitamin-B have already been presented in the discussion of experiments with these preparations on animals fed the ordinary mixed diet. The Vegex preparation was not tested for its vitamin-B content.

SUMMARY: In a dog which has responded to a lack of vitamin-B in its food by a loss of appetite, the secretory function of the pancreas is in no way influenced by intravenous injection of vitamin-B; however, the pancreas of such an animal responds to a small amount of secretin in a characteristic fashion by a vigorous flow of secretion.

The flow of pancreatic juice, bile and saliva in polyneuritic dogs. Three experiments were performed upon polyneuritic dogs to determine the effect of injections of vitamin-B preparations upon the secretory functions of such animals. The dogs, in which polyneuritis had developed, were anesthetized, the various ducts were cannulized, and the different vitamin-B preparations were injected intravenously.

The pancreas and salivary glands of polyneuritic dogs are not influenced by intravenous injection of vitamin-B.—Typical protocols follow.

PROTOCOL VII. *Experiment 26.* Dog 26, female, polyneuritic after 41 days feeding on vitamin-free diet, weight 9.3 kilos, given 1.7 gram chloretone by stomach sound. Cannulae in ducts from right submaxillary and sublingual glands; injection cannula in femoral vein.

Chorda tympani stimulated; copious flow of saliva ensues.

10:15–10:43. Injected 50 cc. *Osborne-Wakeman yeast fraction* solution (= 5 grams of dried yeast)
10:15–10:49. No flow of saliva
10:49. Chorda tympani stimulated: flow of saliva
10:51–11:11. Injected 20 cc. *Rice Polish II* (= 40 grams)
10:51–11:41. No flow of saliva
11:41–11:55. Injected 23 cc. *Wheat Embryo III* (= 30 grams)
11:41–12:30. No flow of saliva
12:30. Chorda tympani stimulated: flow of saliva
12:36–12:49. Injected 20 cc. *Navy Bean I* (= 40 grams)
12:36–1:28. No flow of saliva
1:28–1:46. Injected 30 cc. *tomato juice* neutralized 2 days before
1:28–2:18. No flow of saliva
2:18. Injected 1 mgm. of *pilocarpine hydrochloride*
2:18–2:31. 49 drops saliva from submaxillary gland

PROTOCOL VIII. *Experiment 27.* Pancreatic fistula also prepared on dog 26. *Pylorus ligated.*

3:22–3:27. Injected 30 cc. *Navy Bean I* (= 60 grams)
3:27–3:37. Cannula not filled but a slight secretion is shown by movement of fluid along cannula
3:37–3:41. Injected 30 cc. *tomato juice* neutralized 2 days before
3:37–3:53. Cannula filling at same rate; no apparent effect of the injection on flow
3:53–3:56. Injected 0.2 gram *Osborne-Wakeman vitamin fraction* from yeast (= 2 grams dried yeast)
3:52–3:57. Cannula nearly filled; no apparent effect of injection on flow
3:57–4:02. 1 drop of pancreatic juice

4:02–4:04. 0 drop
4:07–4:12. 0 drop
4:12–4:15. Injected 15 cc. *Wheat Embryo IV* (= 30 grams)
4:12–4:17. 1 drop of pancreatic juice
4:17–4:37. 0 drop
4:37–4:42. Injected 20 cc. *Rice Polish II* (= 40 grams)
4:37–4:47. 1 drop of pancreatic juice (at 4:44)
4:47–4:57. 0 drop
4:57–5:22. 1 drop (at 5:21)
5:22. Injected 1 mgm. *pilocarpine hydrochloride*
5:22–5:47. 7 drops of pancreatic juice

1 cc. Rice Polish II contained 0.128 gram solids.
1 cc. Wheat Embryo III contained 0.153 gram solids.
1 cc. Navy Bean I contained 0.040 gram solids.
1 cc. Tomato juice preparation contained 0.062 gram solids.
1 cc. Osborne-Wakeman yeast solution contained 0.010 gram solids.
1 cc. Wheat Embryo IV contained 0.148 gram solids.

In another experiment on a polyneuritic dog, where the effect of vitamin-B on the rate of flow of pancreatic juice and bile was determined, likewise only negative results were obtained.

The doses of the different products used in these experiments were based upon the amounts of the same products which had been found effective in restoring appetite to dogs or in having a distinct therapeutic effect in polyneuritic animals.

SUMMARY: Vitamin-B has no direct influence upon the secretory function of the pancreas, liver, or salivary glands in an animal which has been fed on a diet lacking this essential.

THE OCCURRENCE OF SECRETIN IN THE INTESTINAL MUCOSA OF ANIMALS WHICH SHOW SYMPTOMS RESEMBLING POLYNEURITIS. As additional evidence that vitamin-B may be identical with secretin, Voegtlin and Myers (1919) cited the fact that they were unable to demonstrate the presence of secretin in the intestinal mucosa of a polyneuritic cat. Anrep and Drummond (1921) performed some experiments to test this point and obtained the opposite result. During the course of the present investigation the intestines of many polyneuritic dogs became available, and therefore tests were made to determine whether or not secretin is present in the intestinal mucosa of such animals. Experiments were made with such material from eight different dogs; four of these never received any treatment with vitamin-B; two were polyneuritic subjects upon the secretory glands of which the effect of different vitamin-B preparations had been determined; the remaining

two animals had received treatments with vitamin-B without any relief from the symptoms.

Secretin is present in the intestinal mucosa of animals which show symptoms resembling polyneuritis. The following protocol is evidence for this statement.

PROTOCOL IX. *Experiment 42.* Dog 25, female, weight 8.5 kilos, given 0.040 gram of morphine sulphate subcutaneously and 1.7 gram chloretone by stomach sound. Cannulae in pancreatic duct and facial vein; pylorus ligated.

11:51.	Injected preparation from polyneuritic dog (no. 11), which had severe convulsions that were only slightly influenced by administration of vitamin-B
11:52–12:01.	36 drops of pancreatic juice
12:01–12:11.	24 drops
12:11–12:21.	20 drops
12:21–12:31.	8 drops
12:31–12:36.	2 drops
12:36.	Injected preparation from polyneuritic dog (no. 12), which had died suddenly during a convulsion and had not received treatment with vitamin-B
12:36–12:46.	99 drops of pancreatic juice
12:46–12:56.	49 drops
12:56–1:06.	12 drops
1:06–1:11.	2 drops
1:11.	Injected preparation from polyneuritic dog (no. 19) which had never received treatment with vitamin-B
1:11–1:21.	86 drops of pancreatic juice
1:21–1:31.	47 drops
1:31–1:41.	14 drops
1:41–1:46.	4 drops
1:46–1:51.	2 drops
1:51.	Injected preparation from polyneuritic dog (no. 20), which had been operated upon and injected with different vitamin-B preparations
1:51–2:01.	79 drops of pancreatic juice
2:01–2:11.	54 drops
2:11–2:21.	20 drops
2:21–2:26.	3 drops
2:26.	Injected preparation from polyneuritic dog (no. 21) which had died suddenly during a convulsion and which had never received treatment with vitamin-B
2:26–2:36.	66 drops of pancreatic juice
2:36:2:46.	32 drops
2:46–2:56.	7 drops
2:56–3:01.	3 drops
3:01–3:05.	2 drops

3:05.	Injected preparation from polyneuritic dog (no. 23) which had received treatment without relief of symptoms
3:05–3:15.	68 drops of pancreatic juice
3:15–3:25.	43 drops
3:25–3:35.	14 drops
3:35–3:40.	3 drops
3:40.	Injected preparation from polyneuritic dog (no. 26) which had been operated upon and injected with different vitamin-B preparations
3:40–3:50.	63 drops of pancreatic juice
3:50–4:00.	40 drops
4:00–4:05.	8 drops
4:05–4:10.	4 drops
4:10.	Injected 5 cc. of preparation from polyneuritic dog (no. 24), which had died suddenly and had never received treatment with vitamin-B
4:10–4:15.	8 drops of pancreatic juice
4:15–4:20.	4 drops
4:20–4:25.	3 drops
4:25.	Injected 10 cc. preparation from dog no. 24
4:25–4:30.	16 drops of pancreatic juice
4:30–4:35.	6 drops
4:35–4:40.	4 drops

SUMMARY-CONCLUSION

A number of solutions, such as extracts of rice polishings, wheat embryo, navy bean and yeast, were shown to contain vitamin-B by tests upon polyneuritic animals (dogs and pigeons).

These solutions were then tested for their possible action on the secretory function of the pancreas, liver and salivary glands. The effect of the products on the rate of flow of pancreatic juice and bile was noted in anesthetized dogs, in which the pylorus was ligated to prevent secretion due to discharge of acid chyme from the stomach, and the discharge of gall bladder bile was prevented by ligation of the cystic duct. Fresh secretin solutions prepared by the usual method were injected for comparison. Each product was also tested for its possible action on the secretory function of the salivary glands of anesthetized dogs in which the ducts from the submaxillary and sublingual glands were cannulized. Stimulation of the chorda tympani nerve and the injection of pilocarpine served as control procedures in such experiments. The effect of the products on secretory glands was noted in dogs which had subsisted on a normal mixed diet and dogs which had been fed a diet free from vitamin-B.

All of these products demonstrated to contain vitamin-B were without any noticeable effect on the rate of flow of pancreatic juice, bile and saliva.

The intestinal mucosae from eight polyneuritic dogs were examined and found to contain secretin.

There is no direct relation between vitamin-B and the secretory function of the pancreas, liver and salivary glands. The hypothesis that vitamin-B functions to stimulate these glands to secretory activity is not supported by the experimental results obtained in this investigation.

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STUDIES OF THE SUGAR IN THE BLOOD OF PIGEONS

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While it is a recognized fact that much of our experimental data in physiology must be obtained from animals other than man, there has been very little hesitation on the part of many experimenters in drawing conclusions concerning phenomena in man from data derived from other animals. In many cases the results have warranted the practice, in others disappointment has resulted. Actually the larger the number of experiments performed, and the greater the number of species from which the data have been derived, the more justification there is for a generalized statement, and for its application to species other than those upon which work has been done. With this idea in mind it was thought wise to make the determinations leading to the data published in the present paper, on an animal hitherto little used in metabolism work. A bird rather than a mammal was chosen largely because of the fact that in this form the red blood corpuscles are nucleated. That this makes a striking difference in the physiology of the blood is well illustrated by Warburg's (1) work on oxygen consumption in the drawn blood of birds as compared with that of mammals. The fact that the red corpuscles of birds are nucleated should prove of especial value in studies made on them with the idea of using the results as a means of giving a better understanding of the physiology of the cells of tissues other than blood. A considerable literature has developed upon the exchange of material between the red cells and the plasma. This is especially true of the chlorides and the sugars. The data upon which this literature is based have been obtained from studies on the blood of mammals. It seems probable that parallel studies on bird's blood with its nucleated cells should lead to a better understanding of this process.

The fact that the birds, characteristically, have a higher temperature than mammals is an additional reason why their metabolism should be studied for comparison with that of mammals. In fact, it has been

suggested by Bierry (2) that this is the reason for the very high concentration of sugar found in their blood as compared with that found in the blood of mammals. That there is a correlation between body temperature and blood sugar concentration is shown by the work of Hollinger (3) and others.

Since most of these properties are of general interest the bird should be an instructive addition to our list of laboratory animals. Comparatively little has been done on the metabolism of birds aside from some work on polyneuritis. It is therefore advisable to extend our knowledge of this phase of their physiology.

From birds in general the pigeon was selected and it is thought that it should be a practical and convenient bird with which to work for the following reasons: *a*, it is of convenient size; *b*, it is comparatively easy to obtain; *c*, its first cost is not prohibitive; *d*, it is easily and economically kept; *e*, it is a seed eater and therefore herbivorous. Further, in the use of the carrier pigeon there is an opportunity for the study of fatigue. Mosso (4), in fact, has already made some use of it for this purpose. This phase of the work we hope to extend, and consequently further data on resting birds are necessary for comparison with those obtained from fatigued birds and from resting and fatigued mammals.

METHOD: *1. The care of the birds.* The pigeons were confined in the laboratory until they became accustomed to their surroundings. They were subjected to frequent handling in order to reduce their fear of the operator, and so to minimize as far as possible results which might arise from fright. In most cases the birds wholly ceased to resist handling when taken from the cage. During periods between experiments the pigeons were kept in a large enclosure giving them opportunity to fly about freely. The food consisted of a mixture of corn, oats, barley and some other grains, and a plentiful supply of fresh water. Access was given to both food and water at all times not otherwise noted.

2. Method of estimation of sugar. MacLean's (5) micro-method was used throughout the research. The macro-method had been successfully used in this laboratory for other blood sugar determinations. The micro-method possesses the obvious advantage of requiring only a small quantity of blood (0.2 cc.). It therefore may be used for animals having a comparatively small amount of blood, and it permits of drawing consecutive samples at relatively short intervals, without producing serious effects from hemorrhage. The extreme limits of error

were found to be about 10 mgm. per 100 cc. of blood. Variations of this amount or less may, therefore, be disregarded.

3. Special technique. While the samples of blood were being drawn the birds were encased in a strong cloth jacket made especially for the purpose. This was done as a matter of convenience to the operator and of safety to the birds. The jacket laced across the ventral surface of the body in such a manner as to be adjustable to birds of varying size, and to secure the wings and legs.

The blood was drawn directly from the heart into a 0.2 cc. pipette by means of a long hypodermic needle. This needle was inserted at the end of the breast bone, going directly through the skin and body wall into the abdominal cavity. The tip of the needle followed the breast bone up to the region of the heart, and so avoided puncture of the liver and other viscera. When the heart could be felt at the tip of the needle the point was dropped slightly and plunged into the heart tissue. Following this procedure the needle struck the heart in such a way that arterial blood was drawn. Post-mortem examination showed that the needle usually entered the heart near the apex on the left side. Hypodermic needles, 20 gauge, and 3 inches long, were used most successfully. Since the distance from the tip of the breast bone to the heart is very nearly equal to the length of such a needle, it is obvious that this method of drawing blood would not be practicable for birds much larger than the pigeon. It is also probable, because of the lesser quantity of blood and the smaller and more delicate heart, that this method would not be applicable to species much smaller. All the experiments were performed during the fall and winter of 1920–21.

STUDY OF THE CONCENTRATION OF SUGAR IN THE BLOOD OF INDIVIDUAL BIRDS. It is a common practice in physiological laboratories to keep the experimental animals for only a short time. While there are probably many cases where repeated observations have been made on individuals, they have not been published with this point in view, and as a result it is difficult to find data showing whether or not the concentration of the sugar in the blood is approximately constant, or whether it is markedly variable from time to time in one animal, and from individual to individual.

In order to determine these points for the pigeons, two were set apart to be used exclusively for this study. They were kept in the laboratory throughout the period of observation and had access to food and water at all times. The observations were extended from

October 29, 1920 until the death of pigeon A on February 20, and until March 13, 1921 on pigeon B. The first six observations were made at intervals of one week. Following this the intervals were lengthened as indicated in table 1.

From this table it will appear that pigeon A, in all but four of the eleven determinations, had a sugar content of 175 mgm. or 180 mgm. per 100 cc. of blood; pigeon B, with four exceptions, 170 mgm. or 175 mgm. per 100 cc. These variations are well within the limits of error for the method, and so are to be considered constant.

TABLE 1

Blood sugar of normal birds

DATE	A		B	
	Weight	Glucose per 100 cc.	Weight	Glucose per 100 cc
	grams	*mgm.*	*grams*	*mgm.*
October 29	317	200	368	140
November 5	325	175	372	170
November 13	327	175	375	170
November 20	330	180	380	175
December 5	330	150	377	215
December 11	335	180	375	175
December 18	335	175	370	170
January 8	337	180	375	170
January 15	340	175	377	175
February 6	337	185	375	165
February 20	342	195	377	190
March 13			375	175

Obviously, then, there is a concentration of sugar which is characteristic of the blood of a given bird. The fact, however, that the two birds which happened to be selected yielded characteristic values so close together, does not warrant the extension of this value to other birds, as will appear later. But while it is evident that there is a value which is characteristic of a given bird, it is also evident that this value will not necessarily be obtained at all times. The occasional striking variations shown in the table illustrate the fact that here we are dealing with an organism which is responsive to modified conditions, and one of its means of adjustment to its environment involves changes in the concentration of blood sugar. This offers experimental confirmation of the statement of Pike and Scott (6) that the concentration of sugar in the blood is one of those internal conditions existing

in higher organisms which are generally constant, and which are regulated by the general nervous and physico-chemical mechanisms of the organism as a whole.

Three cases offered an opportunity for comparing the blood sugar concentration given by individuals at various times after a period of 48 hours' inanition. These data are given in table 2, from which it will be observed that each bird has a value which appears to be characteristic for it.

TABLE 2

Blood sugar of individuals after inanition

BIRD	DATE	BLOOD SUGAR PER 100 CC.	
		Normal	After inanition
		mgm.	*mgm.*
5	10–11	190	165
	12–17		175
9	10–22	185	165
	1– 7		185
20	11– 4	140	100
	12–18		135

TABLE 3

Blood sugar of normal dogs at different times

DOG	DATE	BLOOD SUGAR PER 100 CC.
		mgm.
A	11– 6	75
	1– 6	73
C	10–31	67
	11– 7	65
E	11–20	59
	1–16	63

These results are similar to those reported by Scott and Hastings (7) for dogs. Two determinations each were made on three different dogs, the greatest difference between consecutive determinations upon the same dog being 4 mgm., as shown in table 3.

In a series of 22 blood sugar determinations made by Kramer and Coffin (8) on a quiet dog, the amount of glucose varies from 87 mgm. to 93 mgm. per 100 cc., the average being 89 mgm. per 100 cc.

Jones (9) states that in making repeated observations on individual rabbits the variations were within the experimental error, and so could be considered as constant. She frequently found, however, a considerable variation in passing from individual to individual.

In a recent paper Strouse (10) reported a series of observations on five normal persons covering a period of 8 months. In the series the variations for individuals range from 27 mgm. to 59 mgm. per 100 cc. of blood with an average variation of 41 mgm. per 100 cc. Thallinger (11) made repeated observations on a boy with furunculosis, whose blood sugar was abnormally high, varying from 145 mgm. to 155 mgm. per 100 cc. on a liberal diet which was low in carbohydrates.

Thus it will be seen that the pigeons agree with the rabbits and dogs in possessing a characteristic sugar value. Strouse's figures would

TABLE 4

Effect of excitement on blood sugar

BIRD	DATE	BLOOD SUGAR PER 100 CC.	REMARKS
		mgm.	
4	10- 9	185	
	1- 7	265	Excited by presence of several strangers
8	10–22	185	
	10–22	300	Excited by loud talking
10	11–19	175	
	11–20	250	Excited by escape from cage

indicate that the range may be greater in man, although it is not clear whether this greater range is due to a more ready response to changes in the environment than occurs in the other animals studied, or whether the sugar-controlling mechanism is not so perfectly developed, or whether possibly the conditions of living were not so carefully standardized

EFFECT OF HANDLING; EMOTIONAL GLYCEMIA. As noted in the foregoing, noise, loud talking and the presence of strangers produce a rise in the blood sugar. Rough, sudden or uncertain handling also disturbs the pigeon and increases the blood sugar. In one instance the bird escaped from the cage just before a sample was drawn and some confusion attended its recapture. The amount of sugar in the sample of blood was high, as shown in no. 10, table 4.

From this table it will be seen that the bird offers no exception to the principle long ago pointed out by Boehm and Hoffman (12), Pavy (13) and others, and more recently by Cannon (14), Shaffer (15) and Scott (16), that to obtain blood sugar figures of value, samples of blood must be obtained without pain or other emotional disturbance of the subject. The fact, however, that birds which were known to be excited have such high figures as appear in table 4, indicates that the lower figure of about 185 mgm. per 100 cc. may be assumed to be normal. This was the value obtained by Scott and Honeywell (17) and though Fleming (18) found a much lower value for ducks, in fact a figure quite comparable with that characteristic of mammals, the normal value for the pigeon, at least, appears to be much higher and to agree well with the values published for other birds (cf. Scott and Honeywell).

EFFECT OF INANITION. For reasons which will be discussed later, the birds were subjected to a 48-hour period of inanition in determining the alimentary glycemia curve to be described in the following section. In all cases the concentration of sugar in the blood was determined soon after the arrival of the birds at the laboratory, and again at the close of the 48-hour fast and just before feeding the glucose. While the effect of this inanition was not the primary purpose of the experiment, this procedure offered opportunity for its study, provided that the initial values can be taken as normal values for birds on full feed. The propriety of this is in some doubt, as the birds had not yet become fully accustomed to their new environment and had not been subjected to standard conditions. This would probably result in rather wide variation from values characteristic for the individual with a general tendency to yield high values. It is felt, however, that the figures as they stand merit some attention.

As noted by Rogers (19), the general effect of inanition on the normal pigeon is to increase its natural restlessness. It becomes irritable and fights on the slightest provocation, such as a sudden noise. This might lead one to expect higher blood sugar values in birds subjected to inanition, and may explain those values which are even higher than normal that were occasionally found.

From table 5, which contains the data for the blood sugar of pigeons after inanition, it appears that in 36 experiments 19 pigeons show a lower blood sugar after inanition than before. The results vary from 3.1 per cent to 70 per cent below the initial value. Fifteen pigeons exhibited an increase in blood sugar ranging from 5 per cent to 100 per cent. Two pigeons showed no change whatever. The entire series

TABLE 5

Effect of inanition

BIRDS	DATE	PERIOD OF INANITION	WEIGHT			BLOOD SUGAR PER 100 CC.		
			Before inanition	After inanition	Per cent of loss	Before inanition	After inanition	Per cent of difference
		hours	*grams*	*grams*		*mgm.*	*mgm.*	
10	November 17 to 19	48	280	250	10.7	200	175	− 8.00
11	November 17 to 19	48	350	320	8.5	175	120	−31.0
12	November 17 to 19	48	300	240	20.0	155	150	− 3.1
13	December 15 to 17	48	300	280	6.6	160	100	−37.6
14	December 15 to 17	48	310	275	11.3	105	90	−14.2
5	December 15 to 17	48	340	325	4.4	190	175	− 7.8
9	January 5 to 7	48	280	272	2.8	140	185	32.0
17	January 5 to 7	48	325	312	4.0	185	265	43.0
18	January 5 to 7	48	300	290	3.3	175	310	77.0
19	January 5 to 7	48	350	330	5.6	150	290	93.0
20	November 2 to 4	48	280	248	11.4	140	100	−28.0
21	November 2 to 4	48	310	280	9.65	120	105	−12.5
22	December 8 to 10	48	330	305	7.6	200	170	−15.0
23	November 10 to 12	48	340	310	8.8	155	155	0.0
14	November 10 to 12	48	330	315	4.5	105	210	100.0
25	November 10 to 12	48	230	215	6.5	105	170	62.0
26	November 10 to 12	48	290	250	13.8	255	155	−39.0
27	December 2 to 5	48	345	340	1.4	120	150	25.0
28	December 2 to 5	48	330	305	7.6	150	140	− 6.6
29	December 8 to 10	48	365	340	6.8	120	175	46.0
8	December 16 to 18	48	330	300	9.1	185	55	−70.0
9	December 16 to 18	48	300	262	12.6	140	135	− 3.5
4	December 16 to 18	48	300	290	3.3	185	100	−46.9
33	December 20 to 22	48	340	330	2.9	160	175	9.4
12	December 20 to 22	48	330	317	3.9	155	155	0.0
11	December 20 to 22	48	300	285	5.0	175	150	−14.2
36	December 19 to 21	48	300	280	6.7	185	110	−40.6
37	December 19 to 21	48	300	285	5.0	190	160	−15.8
38	December 19 to 21	48	380	340	10.5	185	115	−37.9
9	December 19 to 21	48	350	340	2.8	140	180	28.6
2	September 11 to 14	48	220	195	11.3	290	300	3.4
3	September 11 to 14	48	216	200	7.4	190	200	5.2
8	October 20 to 22	48	356	300	15.7	185	200	8.1
9	October 20 to 22	48	360	280	22.2	187	165	−11.7
51	October 20 to 22	48	330	272	17.6	195	205	5.1
52	November 2 to 4	48	340	320	5.9	140	160	14.3
Average						166	165	− 0.6

gave an average decrease in blood sugar of 0.6 per cent. It may, therefore, be that 48 hours inanition has practically no effect on the blood sugar of the pigeon. As pointed out above, the evident irritability of the birds subjected to inanition with its possible effect upon the concentration of sugar in the blood should be borne in mind.

EFFECT OF INGESTION OF GLUCOSE: *1. Special technique and discussion.* As noted in the previous section, the sugar was determined upon the arrival of the birds in the laboratory. Also as described above, after the birds had become accustomed to the laboratory, they were subjected to a fast of 48 hours and the sugar in the blood again determined. The results of these two determinations were given in table 5. In addition to the reasons usually assigned for a preliminary period of inanition, this somewhat prolonged period seemed to be necessary to empty the crop and so to insure a rapid passage of the sugar to the region of the alimentary tract where absorption might be expected to take place. It will be readily appreciated that this is even more essential in the case of such birds as the pigeon, which are provided with crop and gizzard, neither of which is presumably a region of absorption, than it is with the mammals, and possibly than it would be with other birds. The alimentary canal was empty in all birds examined, so this period of inanition may be considered as sufficient to fulfill its purpose.

After the second sugar determination, the appropriate amount of glucose was administered. To facilitate the feeding, the glucose was made into tablets and a weighed amount, 1, 2 or 3 grams, according to the series, was given to each bird. In feeding the sugar, the beak was opened and the tablets were dropped well back into the mouth. If the pellets were not readily swallowed, a little water was given through a dropper. Sometimes gentle stroking of the throat seemed to aid when swallowing was especially slow.

Three series of experiments were carried out. In series I, each pigeon was fed 1 gram of glucose; in series II, 2 grams; and in series III, 3 grams. In terms of grams per kilogram of body weight, the average amount of glucose fed was 4 grams, 7 grams, and 10 grams in the respective series.

The ordinary clinical test for carbohydrate tolerance is 100 grams or about 1.4 grams per kilogram of body weight, if the average weight for man is taken to be 70 kilograms. This amount was fed by Cummings and Piness (20), Hiller and Mosenthal (21), Jacobsen (22), Tachau (23) and Strouse, who has also fed 2 grams and 2.8 grams per kilo to normal men. Jones gave rabbits an average dose of 7.87 grams per kilogram.

From the foregoing it will be seen that the amounts given to the pigeon exceed those usually given man in similar experiments. In spite of this, the smallest dose used in the present experiments which is equivalent to one of 1.75 grams for a man weighing 70 kilograms, had very little effect on the concentration of sugar in the blood of the pigeon. In a man such an amount would in all probability raise the concentration of blood sugar to 200 mgm., and probably induce glycosuria.

2. Time of the maximum. Since it was desired to determine the principal points in the entire curve, that is, to follow the curve to its return to the initial value, and since the number of samples of blood which could be drawn safely in any one experiment was limited because of the injury to the heart which would result from repeated

TABLE 6

Time of maximum of alimentary glycemia

BIRD	GLUCOSE FED	BLOOD SUGAR AFTER INANITION	BLOOD SUGAR PER 100 CC. AFTER FEEDING GLUCOSE				
			1 hour	2 hours	3 hours	4 hours	5 hours
	grams		*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
61	3	160	155	240	400	335	320
62	3	180	185	235	305	210	215
64	2	210	205	245	315	275	260
65	2	185	200	215	240	250	205
66	1	170	190	200	215	240	200
67	1	165	170	185	205	225	190

punctures at short intervals, it was necessary to determine the time elapsing between the administration of the glucose and the maximum sugar concentration in the blood. For this purpose, as indicated in table 6, hourly determinations were made after the sugar was fed. It will be seen from the results given in this table that the maximum may be assumed to occur between the third and fourth hours. This last interval was therefore allowed to elapse after feeding and before drawing the first sample, and the sugar level at this time may be assumed very nearly to represent the maximum attained.

When 3 grams of glucose were fed the maximum occurred at or about the third hour. When 2 grams of glucose were fed the maximum occurred in one case at the third hour and in the other case at the fourth hour. After the feeding of 1 gram of glucose, the maximum occurred at about the fourth hour. From these results, given in table 6, it will

appear that the greater the amount of glucose fed the earlier the maximum will be reached.

In this connection it is interesting to note, as Strouse has pointed out, that a heavy dosage often has the effect in man of delaying the onset of the maximum rather than accelerating it as in the pigeon. There must be, then, some fundamental difference between the carbohydrate economy of the pigeon and that of man.

3. Course of alimentary hyperglycemia. In each case samples of blood were drawn just before the administration of the glucose and again after the lapse of 4, 6 and 24 hours. The results are collected in

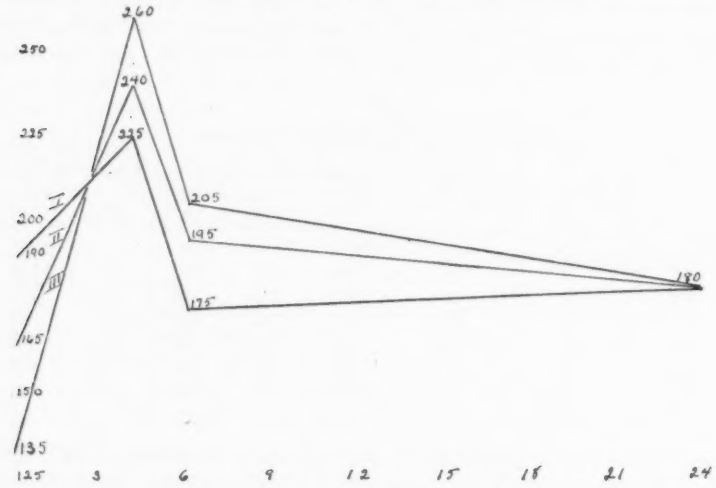

Fig. 1

tables 7, 8 and 9 and summarized in the accompanying curves (fig. 1). In the first series one gram of glucose was given each bird. As noted before, this is about double the ratio of the test meal usually given man for diagnostic purposes. From table 7 it will appear that there is no change or as occurs more frequently, only a slight rise at the end of the first period. The average for the series gives a rise of 18 per cent at this time.

In the second and third series there is a different manifestation. In the second series 2 grams, and in the third 3 grams were given each bird.

The average rise at the end of 4 hours in the second series was 45 per cent, and in the third, 93 per cent. From table 8 it will be seen that

in the second series only five birds had returned to their previous level at the end of 24 hours; and in the third series, table 9, one alone had returned in that interval to the level which existed before the ingestion of the glucose.

While the average at the end of the inanition period may vary somewhat for the different groups, the average at the end of 24 hours after feeding the glucose is approximately that of the normal birds. Because of the relatively low initial values found in the second and the third series, the final values found for these series are distinctly higher

TABLE 7

Effect of ingestion of glucose upon the sugar in the blood

Series I

BIRD	DATE	WEIGHT	GLUCOSE FED PER KILO	BLOOD SUGAR PER 100 CC. AFTER FEEDING GLUCOSE				PER CENT OF INCREASE	REMARKS
				Inanition	4 hours	6 hours	24 hours		
		grams	*grams*	*mgm.*	*mgm.*	*mgm.*	*mgm.*		
10	November 19	250	4.0	175	100	110	250	−37.0	Bird excited by
11	November 19	320	3.1	120	150	150	125	25.0	escape from
12	November 19	240	4.2	155	150	160	150	3.2	cage before
13	December 17	280	3.5	100	275	245	195	175.0	drawing of 24
14	December 17	275	3.6	90	210	300	270	233.0	hour sample
5	December 17	325	3.1	175	170	160	230	− 2.8	
9	January 7	272	3.7	185	270	175	265	46.0	
17	January 7	312	3.2	265	280	275	275	5.7	
18	January 7	290	3.4	310	350	300	250	1.3	
19	January 7	330	3.0	290	295	260	155	1.7	
Average		259	3.9	190	225	175	180	18.0	

than the initial values. Whether or not this is significant we are not prepared to state definitely, although it would seem to be accidental.

Unfortunately there is very little data available which permits of a comparison of the course of the alimentary hyperglycemia of different species. In fact, only three species seem to have been studied from this point of view. In her recent paper, Jones has made determinations on the blood of rabbits only at a single period after the ingestion of the glucose. From the work of Bang (24), the 1-hour period which she chose would presumably give figures at or near the maximum attained. Her results do not, however, permit one to follow the course of the curve. Bang reported a short series of experiments on rabbits which had been

fed from 5 to 20 grams of glucose. After a 5-day period of inanition he found that the maximum was reached in 1½ to 2½ hours, and that in general the sugar level had returned to normal in 6 hours. The amount of sugar did not seem materially to modify the time relations of the curve. The same may be said of a similar but even shorter series, in which sugar was given without a previous period of inanition.

Fisher and Wishart (25) in experimenting with dogs weighing 8 to 9 kilograms, fed approximately 6 grams of glucose per kilogram, and found that the maximum blood sugar occurred one hour after the ingestion of the glucose.

TABLE 8

Series II

BIRD	DATE	WEIGHT	GLUCOSE FED PER KILO	BLOOD SUGAR PER 100 CC. AFTER FEEDING GLUCOSE				PER CENT OF INCREASE
				Inanition	4 hours	6 hours	24 hours	
		grams	*grams*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	
20	November 4	248	8.4	100	150	125	295	50.0
21	November 4	280	7.1	105	300	250	180	185.0
22	December 10	305	6.5	170	290	280	260	70.0
23	November 12	310	6.4	155	440	140	145	184.0
14	November 12	315	6.3	210	210	250	240	19.0
25	November 12	215	9.3	170	200	190	120	17.0
26	November 12	250	8.0	155	250	210	160	61.0
27	December 4	340	5.9	150	280	100	130	87.0
28	December 4	305	6.5	140	200	285	175	103.0
29	December 10	340	5.9	175	270	160	110	54.0
Average		290	6.9	165	240	195	180	45.0

The work of many investigators, notably Cummings and Piness, Hiller and Mosenthal, Hamman and Hirschman (26), Jacobsen and Strouse, indicates that after the ingestion of 100 grams of glucose the maximum concentration of sugar in the blood of man occurs normally in about 30 minutes and that it has returned approximately to its previous level by the end of the second hour. Jacobsen and Strouse point out that occasionally the maximum is attained only after a longer period, and that when this is true the level is apt to be higher than usual, and the return to the previous value is usually slower.

Strouse particularly calls attention to the fact that in diabetes and other conditions which may be presumed to alter the carbohydrate metabolism, such curves are common but that such individuals may be

induced to give the "normal" or usual curve if given less sugar. On the other hand normal individuals will give the "diabetic" curve if the dose be doubled or tripled.

A study of the curves obtained from pigeons shows that the maximum occurs from the third to the sixth hour after feeding, and when amounts were fed which essentially altered the sugar level, the curve did not return to normal for a much longer period, in some cases exceeding 24 hours. Thus they resemble more closely the delayed curves obtained from men rather than the usual or normal one, and, at first thought, the obvious reason is the very heavy dose of glucose given to the birds.

TABLE 9

Series III

BIRD	DATE	WEIGHT	GLUCOSE FED PER KILO	BLOOD SUGAR PER 100 CC. AFTER FEEDING GLUCOSE				PER CENT OF INCREASE	REMARKS
				Inanition	4 hours	6 hours	24 hours		
		grams	*grams*	*mgm.*	*mgm.*	*mgm.*	*mgm.*		
8	December 18	300	10.0	55	215	200	205	291.0	
20	December 18	262	11.4	135	320	150	160	137.0	Excited by presence of strangers when third sample was shown.
4	December 18	290	10.3	100	160	300	220	200.0	
33	December 20	330	9.0	175	335	200	110	91.5	
12	December 20	317	9.4	155	240	210	225	55.0	
11	December 20	285	10.5	150	320	195	175	113.0	Struggled and died during drawing of last sample.
36	December 21	280	10.7	110	225	205	260	104.5	
37	December 21	285	10.5	160	200	265	180	65.5	
38	December 21	340	8.8	115	265	110	195	130.4	
9	December 21	340	8.8	180	330	220	210	84.0	
Average		303	9.8	135	260	205	180	93.0	

In order to determine this point, that is, whether the pigeons would respond to a smaller dose and whether or not a maximum occurring during the first hour had been overlooked, four birds were fed 0.4 gram of glucose each, after an inanition period of 48 hours. This is the amount of glucose which, for the weight of the bird, approximates the usual test dose for man. Blood sugar determinations were made 30 minutes, 1 hour and 1½ hours after the ingestion of the glucose. The results are shown in table 10. Since the variations were all within the limits of experimental error, it may be concluded that the pigeon does not respond to as small a dose as does man and that there is a fundamental reason for the difference in the alimentary glycemia curves shown by the two species.

In his series on normal men Strouse obtained an average increase of 42 per cent after an ingestion of 100 grams of glucose, or 1.4 grams per kilogram. The pigeons show an average increase of only 18 per cent after the ingestion of 1 gram or about 3.5 grams per kilogram, and it was not until they had been given 2 grams or 7 grams per kilogram that they approached the percentage increase reported by Strouse for men.

In addition it should, perhaps, be pointed out that the resemblance is more apparent than real for, as mentioned above, the effect of the size of the dose upon the time elapsing between the administration of the dose and the occurrence of the maximum is in the opposite sense in the pigeon and in man. It would thus seem that the mechanism of storage of sugar is somewhat different in the two groups.

Post-mortem examinations of two pigeons which were killed after inanition and before feeding showed that the crop and gizzard were

TABLE 10

Effect of varying amounts of glucose on time of maximum

BIRDS	WEIGHT	AMOUNT OF GLUCOSE FED PER KILO	BLOOD SUGAR IN MGM. PER 100 CC. AFTER FEEDING GLUCOSE			
			Inanition	½ hour	1 hour	1½ hours
	grams	*grams*				
1	250	1.6	180	165	180	175
2	370	1.1	220	225	230	225
3	280	1.4	195	200	190	205
4	350	1.1	185	175	180	175

empty, while the intestine contained only a small amount of fluid. Conditions were the same in pigeons which were examined at the end of the fourth and sixth hour after feeding. The contents of the alimentary tract were not tested for the presence of sugar. Consequently, while the indications as they stand are that the delay in reaching the maximum is not due to delay in absorption, but rather to some peculiarity in the mechanism of storage, one is not justified in definitely drawing such a conclusion until a study of the contents of the alimentary canal has been made in parallel with blood sugar determinations.

Since concentration of the sugar in the bird is normally so high as compared with mammals, in this particular resembling the diabetic, and since the curve obtained from birds somewhat resembles that obtained from diabetic man, there may possibly be some relationship be-

tween the absolute initial height of the sugar concentration and the form of the curve of alimentary hyperglycemia. However, as noted above, it would seem more probable that in the birds the storage mechanism is somewhat different from that common in mammals and that further work must be done before a satisfactory correlation is possible.

SUMMARY

1. Each bird has a characteristic sugar level about which it varies from day to day. In this it resembles the rabbit and dog.

2. These individual variations are caused by variations in the external and internal environment of the bird.

3. A series of inanition values for blood sugar is given and compared with the values found on full diet. From these figures it is concluded that 48 hours' inanition has practically no effect on the blood sugar of the pigeon.

4. It has been found that, in general, when from 1 to 3 grams of glucose are fed to the pigeon the maximum rise in the blood sugar occurs in 3 to 4 hours.

5. It is indicated that the greater the amount of glucose given the earlier will the maximum be reached.

6. When 1 gram of glucose or less is fed to pigeons there is very little modification of the sugar in the blood. When 2 or 3 grams are fed there is a manifest rise in the blood sugar which gradually approaches its former level.

The author wishes to acknowledge her indebtedness to Prof. E. L. Scott for his assistance in the execution of this work.

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STUDIES ON THE VISCERAL SENSORY NERVOUS SYSTEM

IX. The Readjustment of the Peripheral Lung Motor Mechanism After Bilateral Vagotomy in the Frog

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As a sequel to the work on gastric tonus, the above investigation was undertaken to determine whether the hypertonic condition observed by Carlson and Luckhardt (1) in the lung of the frog after vagal section is permanent or temporary. These authors have shown in crucial experiments (1 to 2 hours) that destruction of the medulla or section of the vagi in the frog leads immediately to a permanent hypertonus or incomplete tetanus of the lung neuromuscular mechanism, which makes the lung practically non-functional and useless as a respiratory organ. The question here arises, does this hypertonus, tonus or tetanus persist in the surviving animal, or is there a process of physiological readjustment of the peripheral lung motor mechanism which comes into play similar to the readjustment that gradually takes place in the case of the gastro-neuromuscular mechanism of the bullfrog (2) after section of the vagi or the splanchnic nerves? This would appear reasonable since it must be remembered that the lung is a diverticulum from the foregut (esophagus).

It is interesting to note that Panizzi (3) over seventy-five years ago made observational studies on the external respiratory movements of the frog after complete ligation of the glottis. These animals lived for a period of 21 days when kept at a temperature of 7° to 8° and they continued to make swallowing movements which led him to conclude that swallowing is a part of the action of the respiratory mechanism in breathing, as has been shown by later investigators. This author further states that when the animals are observed some hours after such an operation, ordinarily the flanks are puffed out which one would say was due to the dilatation of the lungs; but this is not so,

for the air has been, on the contrary, forced into the intestinal canal and into the urinary bladder which it distends. However, he did not study the lung mechanism after section of the vagi.

Mochi (4) claims that the lungs remain permanently collapsed and empty even when only the brain in front of the medulla is removed, but this is probably incorrect, since it has not been substantiated by the work of Langendorff (5), Martin (6) and others who have shown that mere decerebration does not alter the respiratory movements and this has again recently been reaffirmed by the work of Carlson and Luckhardt (1). It is further stated by Martin that complete destruction of the brain and cord leaves the lungs entirely empty of air but he does not explain the true lung reaction or indicate in any way that it is due to a persistent hypertonus. Babák (7), in a subsequent paper, quotes several authors as having shown that after vagi section or lung extirpation the frog swallows air, periodically, into the stomach and that this air may actually escape by the cloaca. According to Nikolides (8), (9), section of either the vagi or laryngeal nerves slows the respiratory movements making them at the same time in the former case at least irregular and stronger. Berti and Marzemin (10) report that no lung respiration occurs after section of the vagi central to the giving off of the superior laryngeal nerve but section of the vagi peripheral to this leads to irregular lung respiration on elevation of the temperature. Heinemann (11), one of the older investigators, is accredited with the observation that section of both vagi leads in the course of several days to such abnormal filling of the lungs that some of the viscera are pushed out through the cloaca and that on opening the abdomen of these frogs the lungs were found collapsed or only partly filled. Soprana (12) finds that vagotomized frogs breathe slower and deeper, but die more quickly from asphyxia on elevation of the temperature. Pari (13), on the other hand, states that the vagotomized frog is unable to fill the lungs, the lungs remain collapsed for weeks and the air is forced into the stomach.

It is evident, therefore, that the results of these earlier investigators are more or less conflicting and inadequate in themselves to explain the true lung reaction resulting from destruction of the medulla or section of the vagi and needs re-investigation, especially in view of the results of Carlson and Luckhardt in acute experiments.

Experimental procedure. The experiments forming the basis of this paper were made exclusively on the common laboratory frog (Rana pipiens). Healthy, vigorous animals were selected in pairs, one of

which was kept as a control, while in the other, one or both vagi were sectioned in the region of the neck after anesthesia, as described in a previous paper on the bullfrog (14). After recovery, direct observations were made on the visible changes in the contour of the flanks and the external respiratory movements of the animal and compared with that of the normal or control.

During the observational periods covering several weeks and even months in many cases the animals coupled in groups were kept in a large vivarium which was divided into small compartments. This was provided with running water and the animals in all the later experiments were fed on caterpillars and earthworms in order to keep them in first-class condition, for any depression might defeat the object of the experiment. To further control any possibility of depression in these animals resulting from the confinement, the vagi were sectioned in many of the control animals 5 to 8 weeks after the cutting of the nerves in the first animal but these latter animals always reacted in exactly the same manner as the former. In a few instances, in the earlier part of the investigation, the thoracic and abdominal viscera of the vagotomized frogs were exposed and cannulae inserted into the tips of the lungs as described by Carlson and Luckhardt (1) for studying the tonus and contractions of the lungs, but it was found that direct inspection was just as satisfactory in most cases. Finally, at the close of each experiment the animals were autopsied and the condition of the lungs and other visceral organs noted.

THE EFFECT OF PARTIAL AND COMPLETE SECTION OF THE VAGI ON THE PERIPHERAL LUNG MOTOR MECHANISM

1. Visible changes in the contour of the flanks and the external respiratory movements. Animals in which bilateral vagotomy has occurred lose the normal contour of their flanks, which fall in, making the body line straight or even curved in (figs. 1 and 2). In some cases the condition of the lungs in healthy and vigorous animals was determined by direct inspection through an incision in the abdominal wall shortly after section of the vagi in which it was found that the air in the lung cavity was forced out and the lungs contracted down to a solid mass not only rendering them useless as organs of respiration but at the same time reducing very markedly the lung volume, thus permitting the falling in of the flanks. Moreover, the complete section of the vago-sympathetic nerves has destroyed the inhibitory control over the

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 1. Frog 10—*control*, showing the normal contour of the flanks. (After 6 weeks as a control animal bilateral vagotomy was performed. It then lived 247 days and was the only animal in which complete physiological readjustment of the peripheral lung motor mechanism occurred.)

Fig. 2. Frog 10—9 days after bilateral vagotomy, showing complete absence of the normal contour of the flanks. Note the straight body line.

Fig. 3. Frog 30—7 days after unilateral section of the left vago-sympathetic nerve, showing total absence of the normal contour of the flank on the side of the nerve section only. Compare with the normal contour of the flank of the right side.

Fig. 4. Same animal as in figure 2—32 days after bilateral vagotomy, showing a partial physiological readjustment of the peripheral lung motor mechanism. Note the "olive-shaped" prominences.

peripheral lung automatism, leaving it free to exert its full influence without any check on the lungs, hence the lungs pass into a state of hypertonus or lung tetanus to such a degree as to nullify their function for it is assumed that the vagi inhibitory fibers to the lungs are in constant or tonic activity similar to the cardio-inhibitory mechanism in many animals. In addition, unilateral section of the vagus was made on several animals on both the right and left sides, but in all cases there was a loss of the inhibitory control over the peripheral lung automatism on the side of the section only while the opposite side remained perfectly normal in every way, thus showing that the efferent inhibitory action of the vago-sympathetic on the lungs is unilateral (fig. 3.)

It should be rememberd that the technique followed in these experiments for the cutting of the vagi is even more delicate in this small species of frog than in the large bullfrog and that injury at the seat of operation is not unlikely, which may lead to some peculiar and fatal reactions. For instance, if the pleuro-peritoneal membrane covering the lungs is pierced at the base of the lung it results in the production, in the course of a few hours, of an animal resembling a "puffball," for as the animal swallows air it escapes not only into the pleuro-peritoneal cavity due to the hypertonic state of the lungs, but if the injury is extensive it may even escape through the site of the operation and collect under the skin of almost the entire body, which in extreme cases may even double or triple the size of the animal, thus making it so buoyant that it floats readily on the surface of the water. In such severe injuries the distention of the flanks usually begins at once and continues rather rapidly until the maximal is reached 2 to 4 hours after the operation. Such animals are unfit for experimental use and were discarded after autopsy.

In successfully operated animals it is essentially important to become acquainted with certain reactions that are liable to develop during the first few days following the operation of double vagotomy. Ravitsch (15) a number of years ago pointed out that double vagotomy in the frog paralyzes the stomach, and the writer (14) in a recent paper has shown that such an operation on the bullfrog leads to a hypotonic condition of the stomach covering a temporary period of 8 to 9 days. Normally these animals swallow air which enters the lungs under positive pressure, due to the act of swallowing, but after bilateral vagotomy for a temporary period, it is not only possible but probable that most animals, because of the contracted condition of the lungs which is sufficient to

completely obliterate the lung cavity, swallow air which follows the course of least resistance and passes into the stomach. This results in a distention of the organ to a more or less degree depending on the atonic condition of the gastro-neuromuscular apparatus, and perhaps also on the atonic conditon of the esophagus, since Steinach (16) claims that section of the vagi inhibits and destroys permanently the tonus of the esophagus. It would seem, however, from the results of other work that there ought to be at least a partial readjustment of the tonus of the esophagus and this phase of the question is now under investigation.

When the swallowing of air is excessive as occurs in some animals the air may be forced from the stomach onward into the intestine and even into the urinary bladder resulting in a distention of these organs and a puffing out of the flanks of the animal to a degree considerably more than the normal but in no case approaching the "puffball" frog. In some cases air was observed to escape from the cloaca and in a few others even the viscera protruded for a time through the same opening to the exterior It is probable that the similar observations reported by Heinemann (11) and others (7) were due during the act of swallowing to the actual escape of air into the stomach, intestine and bladder because of persistently constricted lungs. This idea is supported by the recent work of Carlson and Luckhardt (1) who state that the contractions of the lungs following complete section of the vagi are powerful enough to develop a pressure of from 30 to 40 mm. of mercury. In one vagotomized animal in which cannulae were inserted into the tips of the lungs and connected with water manometers after closure of the glottis, the lungs were so markedly contracted fifteen days after the operation that they could be but partially distended even when subjected to a pressure of 14 cm. of water and when the attempt was made again to increase the pressure it resulted in the rupture of one of the lungs, although the normal lung of the frog is easily distended to normal size by a pressure of 3 cm. of water. After a period of 3 to 4 weeks or even longer following section of the vagi there is usually little or no air found in the stomach or the intestine with the possible exception of two animals in the series together with those animals in poor condition—starved, moribund, parasitized or otherwise infected, while in recently operated animals up to a period of from 2 to 3 weeks it is more usual to find air in the stomach and intestine in varying amounts than in similarly operated animals of longer standing. In a few animals I have seen the alimentary tract so distended with air 2 weeks after the operation that it resembled a hydrostatic organ of a fish. It is implied

that the poor condition of animals leads to a loss of tonus of the alimentary tract which may also affect the lungs in the same manner although it appears to be to a lesser degree.

The more common phenomenon, however, is the filling of the bladder with fluid which tends to produce the normal or slightly more than normal curvature of the flanks but this may be relieved by the following simple manipulation which empties the bladder and reduces the animal to the normal state for direct observation whenever desired. This procedure consists in grasping the animal around the body with a gentle but firm grip and then extending and flexing the posterior limbs a few times which results in the escape of the fluid from the bladder through the cloaca, as well as any air present, but it does not empty the stomach and upper intestine of air. It is essential that this procedure be followed before each daily observation otherwise no normal standard of measurement would exist from day to day since the state of the flanks would vary from a positive to a negative curvature depending on the fulness of the bladder. The animals soon become accustomed to this procedure and after a few days react very quickly, so that the task of emptying the bladder becomes an easy matter.

The external respiratory movements in the frog consisting of buccal movements, closing of nares, expiration and inspiration or swallowing air, occur in a coördinated and orderly sequence, the buccal or passive movements proceeding rhythmically between the swallowing acts, so that there are several buccal movements between each swallowing act. This is the usual type of buccal movements in the frog although in exceptional cases there may be at times in certain animals a perfect synchrony between these movements and the actual swallowing of air (inspiration). These facts might indicate that the respiratory center in the medulla is anatomically and physiologically identical with the center for deglutition in this animal.

The buccal or passive movements are distinct from the quick respiratory movements of the flanks, the latter being due to the act of inspiration or the swallowing of air which is preceded by the opening of the glottis and the escape of some air into the buccal cavity. This results first in a diminution in the size of the lungs with a corresponding falling in of the flanks, which is then quickly compensated for by a distention of the lungs which, in most animals, show a periodicity similar to the Cheyne-Stokes' type of breathing in mammals. The buccal or passing movements, on the whole, are little affected as a result of sectioning both vagi although in some cases there is perhaps a

slightly slower rhythm with more shallow movements, whereas the quick respiratory movements of the lungs, on the other hand, are completely abolished for a temporary period but these gradually reappear again through a physiological readjustment of the peripheral lung motor mechanism.

2. The readjustment of the peripheral lung motor mechanism after section of the vago-sympathetic nerves. In both unilateral and bilateral section of the vago-sympathetic nerves there is a gradual physiological readjustment of the peripheral lung motor mechanism which usually starts from 12 to 21 days after the nerve section when the lungs begin slowly to distend and with this distention comes a return of the quick respiratory movements of the lungs which at first are very feeble and shallow but which gradually increase to about the normal as the readjustment proceeds. Two methods were used for studying the lungs during the period of the readjustment. The first was that of direct inspection, the second consisted of the introduction of a small glass cannula into the glottis for inflating and testing the air capacity of the lungs. By the method of direct inspection the first changes to occur were slight enlargements at the base of the lungs which slowly enlarged until they assumed the appearance of prominent "olive-shaped" bodies, although the lungs at this time were usually not distended in the pleuro-peritoneal cavity to more than 60 to 75 per cent of the normal (fig. 4). This latter condition was especially shown by the second method with a cannula in the glottis which was used to test the capacity and the amount of distention of the lungs from time to time as a check-up and control method in all the observations. When such a test was made on an animal a few days after vagotomy the lungs were found to be so contracted in healthy animals that it was practically impossible to distend them at all by mouth inflation. After the appearance of the "olive-shaped" bodies, the lung distention or readjustment became slower and in only one animal that lived for a period of 247 days, or a little over 8 months after bilateral vagotomy, can it be said that there was a complete physiological readjustment and a return to the normal lung, as shown not only by its functional activity but also by its normal distention in the pleuro-peritoneal cavity. This was a normal control animal (frog 10, fig. 1) which had been kept as such for a period of 6 weeks previous to the complete section of the vago-sympathetic nerves. The complete physiological readjustment of the lungs in this particular animal occurred at the end of 232 days, or a little over 7½ months. The animal was apparently normal at the time of this

observation, January 26, 1921, and a week later when fed earthworms ate greedily and did not die until February 9, 1921. The animal showed some emaciation but it was not extreme, a condition produced probably by more or less irregular feeding during the winter months due to the extreme difficulty at times in obtaining food. The autopsy confirmed the above observation and since both lungs remained distended it would indicate a complete readjustment of the peripheral lung automatism and no functional regeneration of the vago-sympathetic nerves. Anatomical evidence of regeneration of the vagus was not present. Furthermore, it should be emphasized that on section of these nerves about $\frac{1}{8}$ inch of the nerve was always removed, the ends never being approximated.

The other animals in the series died at an earlier stage, death occurring in most cases at the end of 2 to 5 months, and in all these animals there was only a partial physiological readjustment of the peripheral lung motor mechanism, although the lungs exhibited functional activity to a marked degree which in many cases practically equaled the normal, yet the lung distention in the pleuro-peritoneal cavity was always less than in the normal animal. However, the animals of 5 months standing always showed a greater physiological readjustment of the peripheral lung motor mechanism than those of less duration (2 to 4 months).

It may be concluded from these experiments, especially from the one in which the animal lived for a period of over 8 months as well as from such experiments as are represented by figure 5, that the failure of the vagotomized lungs to contract down to practically a solid mass on death or brain destruction is evidence that the readjustment of the vagotomized lungs is not due to vagus regeneration. Furthermore, the lung readjustment in these long-time experiments is not due to the gradual weakening of the frogs from age and starvation since animals when fed and kept in close confinement react in the same manner after unilateral or bilateral vagotomy as do normal animals which have not been so kept. Carlson and Luckhardt (1) have found and pointed out that frogs in poor condition show little or no lung tetanus even immediately following vagi section. The explanation of the variance of my results with the acute experiments of these investigators is to be found in the longer duration of the experiments and perhaps to my animals being in a more perfect condition, not only at the start but more especially in the later stages of the experiments, for all animals with low resistance usually succumbed in a few days following the operation and were discarded, so that only animals with good

bodily resistance reached the critical stages of the experiments herein described.

It is evident from these long-time experiments that if the animals are well fed and tended they will live almost indefinitely after bilateral vagotomy.

3. Autopsy findings. The autopsies were performed at the earliest possible moment on all the animals and in many cases the heart was still pulsating while this was being done. The autopsy findings were practically in accord with the preceding observations and in several cases the actual measurements of the lungs were taken. The reader's attention is invited to figure 5 in which unilateral section of the vago-sympathetic has occurred on the left side, while the nerve of the opposite side is intact. It will be noted that the air in the right lung has been almost entirely forced out and that the lung is contracted down to practically a solid mass, while on the side of the nerve section there is distention of the lung. The hypertonus or lung tetanus of the right normal lung is the typical reaction that follows section of the vago-sympathetic or destruction of the medulla. Furthermore, when the center in the medulla which executes the impulses for the vagi inhibitory fibers to the lungs becomes paralyzed following the death of the animal the inhibitory control, holding the peripheral motor automatism in check, is removed, and because of the presence of this peripheral lung automatism the lung contracts and passes into a state of hypertonus or lung tetanus. As a general rule, the lungs of the frog are normally contracted after death in healthy and vigorous animals. In the case of the left lung which has undergone a partial physiological readjustment following section of the vago-sympathetic nerve on this side the lung remains distended because the peripheral lung automatism is adjusted to the chronic absence of the central inhibitory control. Moreover, it is not at all certain or clear that the gradual release of the lung from the hypertonus (tetanus) is the same as loss or absence of the peripheral automatism. This phase of the question must be determined by other lines of investigation. All that can be said, however, is that the hypertonus or lung tetanus disappears gradually, but this may mean, in part, a development to head the (peripheral) inhibitory mechanism, as well as a direct depression of the motor mechanism.

The physiological readjustment of the peripheral lung automatism first starts in the basilar portion of the lung and gradually extends toward the apex, but this distention is always more rapid in the transverse than in the longitudinal plane, hence the "olive-shaped" prom-

inences. However, the apex of the lung is the last part to undergo this physiological readjustment and as indicated in figure 5, which is drawn to scale, the apex is still markedly constricted and the constriction usually extends to a somewhat lesser degree along the mesial surface of the lung from the apex. This constriction of the apical portion of the lung was observed in practically all the animals in a healthy condition with the exception of the animal living for a little

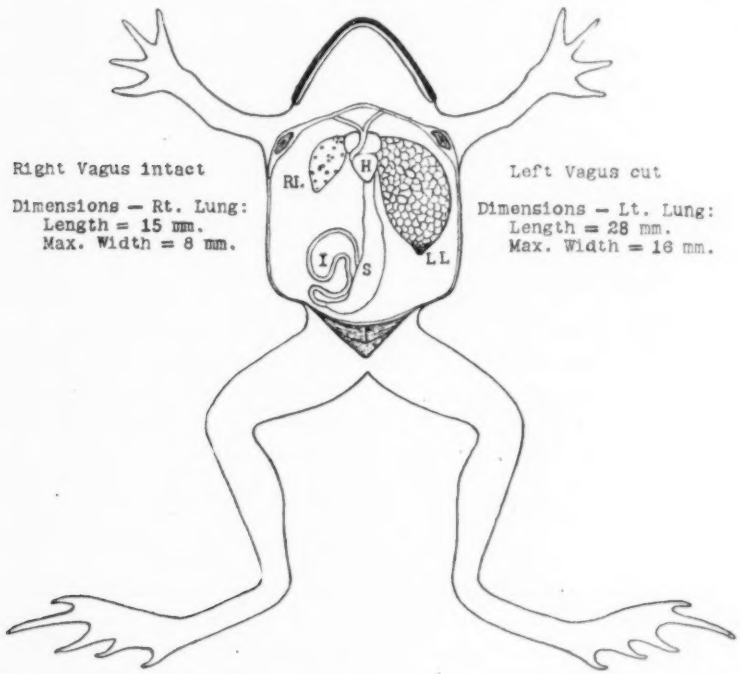

Fig. 5. Frog 17—death of animal 54 days after unilateral section of the left vago-sympathetic nerve, showing that the left lung, *LL*, which has undergone a partial physiological readjustment, remains distended because of readjustment of the peripheral lung automatism. Note the constriction at the extreme apex of this lung. The right normal lung, *RL*, shows the immediate effect of removal of the inhibitory control or check over the peripheral lung automatism; the lung contracts and passes into a state of hypertonus or lung tetanus. For further explanation see text. *H*, heart, *S*, stomach, *I*, intestine. The liver has been removed to show the lungs.

over 8 months in which there was a complete physiological readjustment of the peripheral lung motor mechanism. Therefore it may be implied that the lung may bring about its physiological readjustment through the plasticity of its peripheral neuromuscular mechanism.

CONCLUSIONS

1. Bilateral vagotomy in the frog (Rana pipiens) destroys the inhibitory control over the peripheral lung automatism, leaving it free to exert its full influence on the lungs without any check, hence the lungs contract and pass into a state of hypertonus or lung tetanus to such a degree as to nullify their function. The normal contour of the flanks in these animals disappears and the body line becomes straight or even curved in.

2. In unilateral section of the vago-sympathetic nerve there is loss of the inhibitory control over the peripheral lung automatism on the side of the section only, the opposite lung being unaffected, thus showing that the nerve action is unilateral.

3. In both unilateral and bilateral section of the vago-sympathetic nerves there is a gradual physiological readjustment of the peripheral lung motor mechanism which usually starts from 12 to 21 days after the nerve section when the lung begins to be distended by swallowed air, pushing out the flank and finally forming "olive-shaped" prominences. This readjustment was partial in all the animals with the exception of one, which lived for an extended period of a little over 8 months, the complete physiological readjustment occurring at the end of about $7\frac{1}{2}$ months. In other animals living for periods of from 2 to 5 months, those of 5 months' standing always showed a greater degree of physiological readjustment than those of less duration.

4. In recent bilateral vagotomized animals up to periods of from 2 to 3 weeks air is found more constantly and usually in greater amounts in the stomach and intestine than in similarly operated animals of longer standing. This indicates that the air is forced into the stomach by the act of swallowing because of the persistently constricted lungs, aided probably by a hypotonic stomach, at least in the early stages.

5. The autopsy findings are in accord with the above results.

6. Bilateral vagotomy has little or no effect on the buccal movements, whereas the actual respiratory movements (opening of glottis and swallowing of air into lungs) are temporarily abolished, but these movements gradually return with the physiological readjustment of the peripheral lung motor mechanism.

7. The lung readjustment in these long-time experiments is not due to a gradual weakening of the animals from age and starvation, since animals when kept in close confinement react in a similar manner after unilateral or bilateral vagotomy as do normal animals which have not been so kept. Furthermore, the failure of the vagotomized lungs to contract down to practically a solid mass on death or destruction of the medulla in these experiments is evidence that this readjustment is not due to a vagus regeneration. It may be implied, therefore, that this physiological readjustment of the vagotomized lung is brought about through some special activity of its peripheral neuromuscular mechanism.

The writer desires to express his thanks to Drs. A. J. Carlson and A. B. Luckhardt for helpful and very suggestive criticism.

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STUDIES IN THE DYNAMICS OF HISTOGENESIS[1]

Tension of Differential Growth as a Stimulus to Myogenesis

VIII. The Experimental Transformation of the Smooth Bladder Muscle of the Dog, Histologically, into Cross-Striated Muscle, and Physiologically, into an Organ Manifesting Rhythmicity

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The writer has attempted to prove (1) that function determines structure as regards muscular tissue. The evidence has rested, however, on embryological evidence. For the experimental test the bladder of a young dog was selected, because it possesses the smooth, pale type of muscle. If the various types of muscle, namely, smooth, cardiac and skeletal, represent resultants of different degrees of optimum tension, then the proof would be forthcoming experimentally, if the vesicular non-striated muscle could be transformed into cross-striated muscle by varying the velocity of application and the intensity of the tensional stimulus to a higher optimum degree.

The normal differentiation of cross-striated muscle from the primitive undifferentiated mesenchyme is a gradual one and not a spontaneous act. The heart of the chick embryo, for example, reacts to the tensional stresses induced by the circulation for nearly 100 hours, before cross-striated muscle is formed. The heart begins pulsating about the 30th hour of incubation but cross-striated cardiac muscle is not produced until the 125th to 130th hour. Previously the heart structure is not unlike smooth muscle. What causes the appearance of cross striations? This is a protoplasmic reaction to the amount of work done by the cardiac mesenchyme, the number of contractions made in a unit of time,

[1]This thesis was awarded first prize in the competition for the medical prize scholarships for original research by the Medical Faculty of the University of Chicago, Rush Medical College, 1921.

over a certain temporal interval. These contractions, as regards the heart, are caused by the hydro-dynamic stretching stimulus of the constantly increasing circulatory medium.

These embryological facts caused the writer to reason that the tensional stimulus is the necessary extrinsic factor in myogenesis. The bladder should eventually react by forming cross-striated muscle and by manifesting rhythmicity, if the intra-vesicular hydro-dynamic pressure and volume are increased in degree comparable with that found in the heart.

We are taught at the present time that the essential difference between the types of developed muscle is the presence or absence of cross striations. From the purely static and structural standpoint, these cross bars are the outstanding feature. On the other hand, from the dynamic or functional, embryological view these muscle types represent differences in the amount of *work* that has been done upon the undifferentiated mesenchyme by the differential growing parts of the embryo during the active period of growth. The essential difference, then, physiologically between the various muscles is their capacity for work, which in turn is dependent upon the amount of work that has been expended in their production. The reason for the different degrees of energy possessed by the types of muscles is purely an embryological bio-mechanical problem and corresponds to the differential amount of tensile work that has been expended in their formation by a dominant energetic zone extrinsic to the region of myogenesis.

Experimental results. A shepherd dog 4 weeks old was selected for the experiment. A silver supra-pubic tube was transfixed in the bladder March 30, 1921. To this tube could be attached, when the experiment was in operation, the rubber tube through which fluid passed from a pressure reservoir. In the course from the reservoir to the bladder a mercury manometer was interpolated in order to record the vesicular contractions.

At the initial insertion of the drainage tube, the bladder of this pup presented the pale appearance of smooth muscle. Very little resistance to incision with the scalpel was presented by the bladder musculature. The vesicular wall measured 0.5 mm. in thickness. The bladder of a control puppy 4 weeks old weighed 0.75 gram. At the first operation the bladder was examined histologically; it presented the appearance of typical smooth muscle and possessed transitional epithelium.

Concentrated boric acid 37.5°C. was passed through the bladder under varying volume and pressure conditions and at various intervals

of time daily. On May 21st a second operation was performed for the purpose of excising a portion of the bladder for histological purposes and, if cross-striated muscle were found, to allow the musculature to revert to smooth muscle. The bladder muscle presented a deep red appearance and the resistance to incision with the scalpel had greatly increased. The bladder wall was 5 mm. in width, whereas the bladder in the control puppy was 1.9 mm. The bladder of a control puppy weighed 1.59 grams, whereas the bladder in the experimented dog weighed 4.75 grams.

The volume of boric acid passed by the bladder was gradually increased from April 3rd, 5 days after the suprapubic drainage tube had been inserted in the bladder, until May 21st (see figs. 1 and 2). The volume of the fluid was regulated by means of a screw-clamp and as soon as the puppy showed signs of distress due to vesicular distention the circulation was reduced or stopped completely until the fluid was passed by the urethra. On April 3rd the experiment lasted for ½ hour, during which time 20 cc. of fluid had passed through the bladder and out through the urethra. The urethral passage of urine was of the nature of clonic, short, rapidly recurring contractions. One clonic spasm occurred every 2 minutes and each spasm was constituted of about ten short contractions.

As time went on the clonic nature of the bladder contractions was lost and a greater volume of fluid was passed with each urethral relaxation. Corresponding to each individual contraction of the bladder a complete urethral relaxation occurred. The resemblance of the full contractions of the bladder to that of the heart and the perfect coördination of urethral relaxation and subsequent closure like a cardiac valve gave an ideal experimental condition for inducing in the bladder the circulatory changes found in the heart. A mechanical valve was occasionally used between the elevated pressure reservoir and the mercury manometer. This device gave a still further resemblance of the nature of the vesicular contractions, under a hydro-dynamic stimulus comparable to that found in the heart.

Here was a dog the bladder of which, prior to the operation, March 29th, passed on an average of 250 cc. of urine in 24 hours. On May 20th to 21st this same bladder passed the enormous increase in volume of 50,000 cc. of boric acid during 10 hours of experimental observation. This prolonged increase of intra-vesicular pressure prevented the urine from being excreted and set up a uremic toxemia which so lowered the resistance of the young dog that it died 24 hours after the second opera-

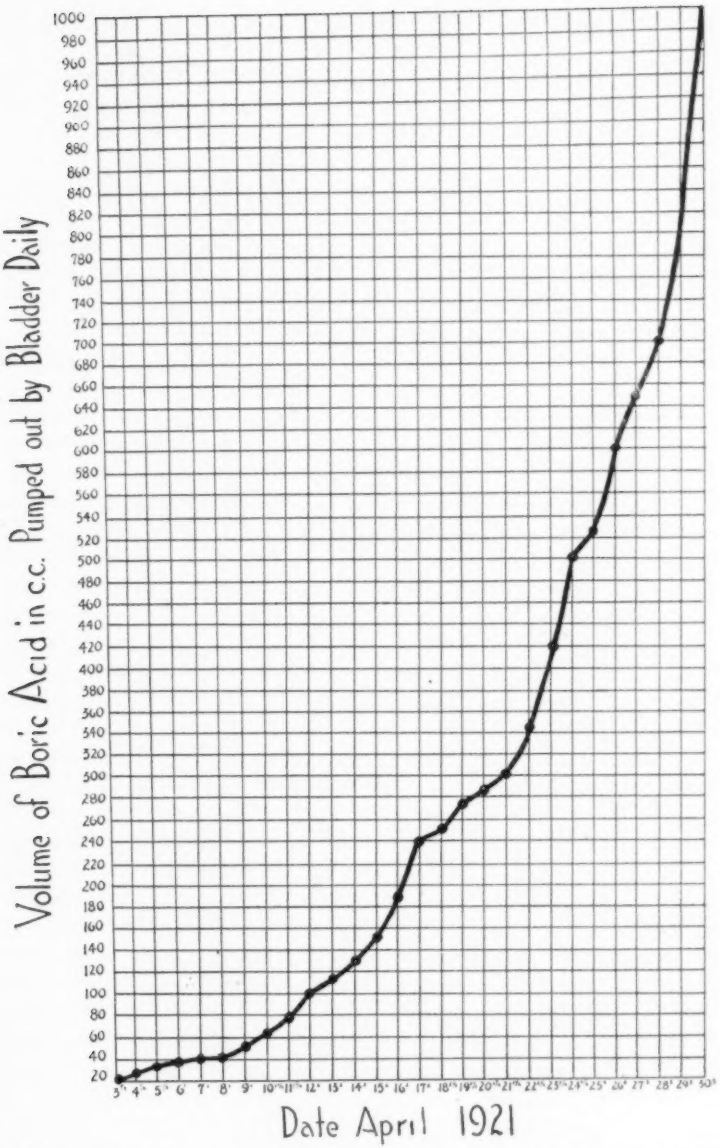

Fig. 1. Curve of volume of urine passed through bladder daily. The small number to the right of each date represents the length of time of the experiment in hours, for each day.

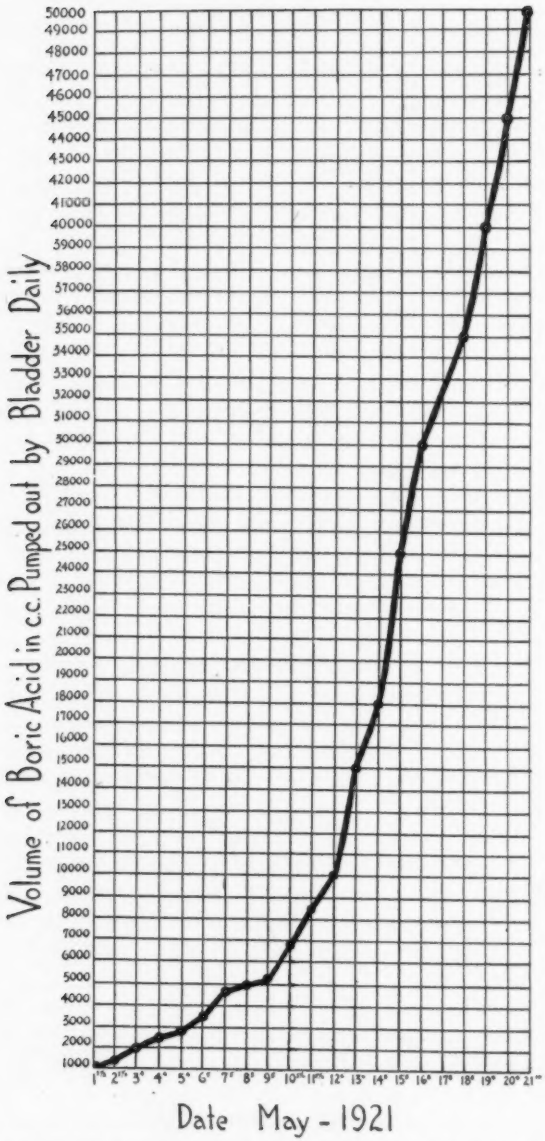

Fig. 2. Curve of volume of urine passed through bladder daily. The small number to the right of each date represents the length of time of the experiment in hours, for each day.

tion. On post-mortem examination both ureters were enormously dilated with urine and measured 10 mm. in diameter. The nephritic pelves were distended with urine and both kidneys were markedly hydronephrotic. The writer intends to have a series of dogs and microscopic demonstrations ready for the next meeting of the Association of American Anatomists at New Haven, December, 1921.

Figure 3 presents the manometric curves from the inlet tube of the bladder during the first hour and 15 minutes of the experimentation May 21st, after the dog had had a night's rest. A constant volume and pressure were maintained throughout the observation. The varying irritability and response of the bladder during this observation are clearly shown. At the beginning, line 1, the bladder is not so irritable as subsequently, lines 2 to 5. At first the contractions are few in number, but as the bladder becomes more irritable to the constant hydro-dynamic tensile stimulus it responds with more celerity.

By reducing the volume and the pressure of the fluid circulating through the bladder the vesicular contractions are retarded; by complete inhibition of the circulation no contractions are elicited (fig. 4, *a* to *b*). The stimulus that causes the rhythmic beat of the bladder is hydrogenic in nature. The rhythmic beat is dependent also on the irritability of the responding mechanism.

Acceleration of the vesicular beats may be induced by increasing the volume and pressure of the fluid flowing into the bladder. By regulating the fluid pressure a condition may be induced whereby the bladder responds vigorously and regularly. When the stimulus was so regulated that 65 to 75 beats per minute were established the bladder reacted with regularity and absolutely no distress was experienced by the puppy. The dog could be maintained in the recumbent position for hours and would breathe regularly—even sleep for a half an hour or so, while the bladder was pumping at the rate of 75 times per minute. There was absolutely no conscious effort on the part of the dog with these rhythmic contractions of the bladder once the experiment was under way and the vesicular irritability had been reëstablished after the dog had had a night's rest. Figure 3 shows the bladder beating at the rate of about 50 per minute during 1 hour and 15 minutes of observation; this record was made with a slow drum. The puppy slept practically throughout the time that these manometric curves were being recorded.

The structure of these pressure curves is definitely seen in figure 5 made on a fast drum. The upstroke represents bladder distention with the concomitant increased back pressure recorded through the inlet tube.

The downstroke represents vesicular contraction, simultaneous with urethral relaxation corresponding to the time that the bladder is being emptied and the concomitant reduction of back pressure through the

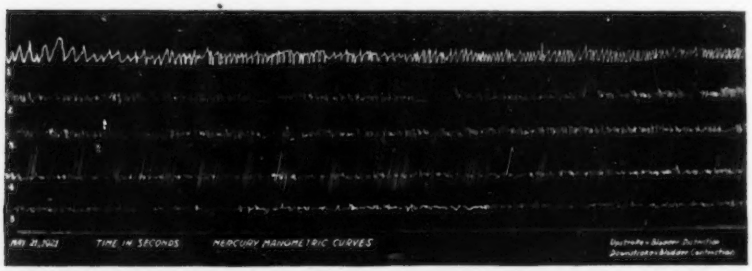

Fig. 3. Lines *1*, *2*, *3*, *4* and *5*, represent the variable response of the bladder to a constant hydro-dynamic tensile stimulus for the first 1 hour and 15 minutes of observation on May 21st, from 7 to 8:15 a.m., after the dog had had a night's rest of 10 hours. At the beginning of the line the bladder made about 6 vigorous contractions a minute, whereas at the end of line *1*, the bladder was contracting at the rate of 30 beats per minute. The average rate in lines *2*, *3*, *4* and *5* is 55 beats per minute. Mercury manometric pressure curves.

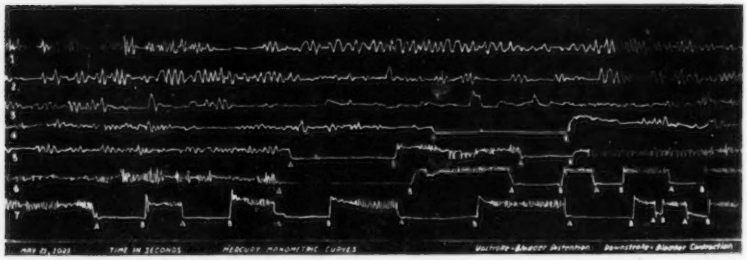

Fig. 4. Lines *1*, *2*, *3*, *4*, *5*, *6* and *7* represent the variable response of the bladder to a variable pressure stimulus. The rate of contraction in lines *1* and *2* average 50 per minute. From *a* to *b*, lines *4*, *5*, *6* and *7*, the fluid circulation was completely inhibited. Concomitant with the lack of the tensile stimulus *a* to *b*, there is a lack of vesicular beats, the pressure drops as noted by the lowered straight line. From *b* to *a* the pressure was suddenly raised. Note the immediate rise in pressure and the acceleration of vesicular beats. The average rate of contraction in line *7* and after the circulation is suddenly established in the bladder is 300 per minute.

inlet tube. These curves were made with the mechanical valve working between the elevated pressure reservoir and the mercury manometer. They are of different types; some are single, others double hill-

ocks, while some show the pressure rising gradually and then a sudden release with the contraction of the bladder. Certain curves show a comparatively acute summit, others a definite plateau. This indicates a variable irritability and response of the modified vesicular musculature to the constant extrinsic tensile stimulus.

The contractions of the bladder musculature are active and independent of the action of the heart and respiration. With each bladder contraction determined by the manometer and by palpation, there is an expulsion of urine. This expulsion is due to vesicular contraction and not to any passive transmission through the bladder by an active zone ex-

Fig. 5. This record shows the structure of the pressure curves on a fast drum. The upstroke represents increased pressure simultaneous with the distention of the bladder; downstroke, decreased pressure concomitant with contraction of bladder and expulsion of fluid through the urethra. These curves are of different types; some are single, others double hillocks, while some show the pressure rising gradually and then a sudden release with the contraction of the bladder. Certain curves show a comparatively acute summit, others a definite plateau. This indicates a variable irritability and response of the modified vesicular musculature to the constant extrinsic tensile stimulus.

trinsic to the contracting vesicle. The abdominal musculature is in a state of normal tonicity and shows absolutely no simultaneous activity with the relaxation of the urethra, bladder contraction and fluid expulsion.

With over-distention of the bladder the dog experienced forced respiratory movements. These, naturally, had their immediate effect on the vesicular pressure just as they do normally. The optimum bladder rhythm was an independent series of contractions, however, which was not caused by any indirect influence from the respiratory or cardiac regions. The elongated respiratory waves upon which the cardiac rhythm is superimposed in normal pulse tracings are obtained.

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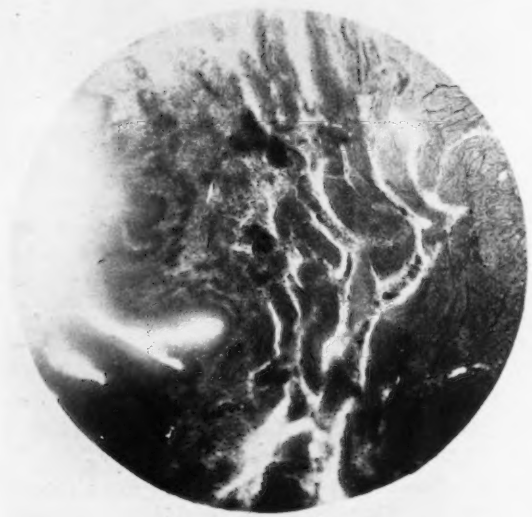

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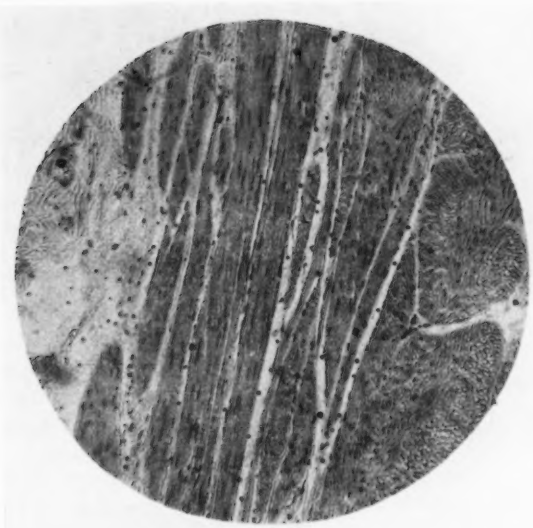
Fig. 6. Microphotograph of bladder prior to experiment. The characteristic arrangement of the smooth muscle and the transitional epithelium are clearly seen. × 70.

Fig. 7. Microphotograph of same bladder 48 days after experimentation. The bladder was subjected to 200 times more work than it normally experiences. The smooth muscle was transformed into striated muscle in response to increased hydro-dynamic tensional stimuli. The transitional epithelium hypertrophied into the stratified squamous type. (× 70 B. and L. objective, 16 mm. 0.25, ocular 5.)



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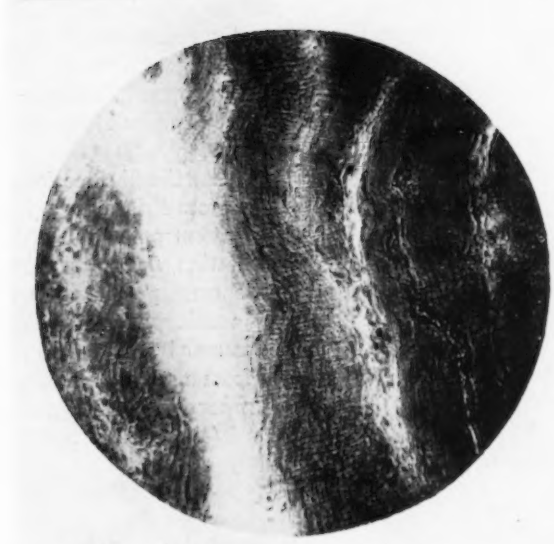

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Fig. 8. Microphotograph of smooth muscle of the bladder prior to the experiment, March 30, 1921. No cross-striated muscle is seen; × 285.

Fig. 9. Microphotograph of the transformed cross-striated bladder muscle May 21, 1921. (× 285, B. and L. obj., 4 mm. 0.85, ocular 5.)

The excised portion of the bladder taken at the initial operation was normal smooth muscle of a developing bladder (see figs. 6 to 8.) The part of the bladder taken at the second operation for sectioning and study showed definite cross-striations and an increase in width and length of the muscle fibers over that of the control (see figs. 7 to 9). The physiological observations of a bladder that had been developed carefully, to respond to increased work two hundred times greater than that which it was normally subjected to, structurally reacted to do this work by the transformation of the smooth young muscle cells into striated muscle cells. The former type of muscle is incapable of doing the work done by the bladder under observation; the latter type possesses the capacity under the requisite stimulus for prolonged rhythmic contractions and increased work. The different degrees of energy possessed by the vesicular smooth and cross-striated muscles here studied is purely a bio-mechanical problem corresponding to the differential amount of work that has been expended in their formation.

The transitional epithelium of the bladder has undergone a hyperplasia and appears as stratified squamous epithelium (fig. 7). There are from ten to thirty layers of cells in the vesicular epithelium. The inner group of cells is greatly flattened and elongated; in certain locations no nuclei are seen in the layer bounding the lumen. In the greater part of the epithelium of the bladder the cells are nucleated from the basal to the inner group of cells.

Bardeen (2) and others have observed that the fibrillae found in developed skeletal muscle at first show no cross-striations The deeply staining segment corresponds with the Q anisotropic band of the adult fiber and the other with the I isotropic striation, Lewis (3). The condition of the cross-striated bladder muscle corresponds to the granular alignment which forms, subsequently, the continuous Q bands illustrated during the development of cross-striated muscle by Godlewski (4). The vesicular cross-striated fibers are formed from a syncytium composed of endoplasmic nucleated units. This is comparable to the developmental observations of McGill (5) and Godlewksi (4) for smooth and striated muscles, respectively.

CONCLUSIONS

1. The evidence herein presented proves definitely that the pale bladder musculature may be transformed histologically into the red, cross-striated type by increasing the tensional stimulus to a degree comparable with that which the cardiac mesenchyme experiences normally and phy-

siologically, into an organ manifesting rhythmicity as long as the hydrodynamic stimulus is applied.

2. The essential difference between the pale smooth muscle of the bladder and the red involuntary striated muscle of the heart is dependent upon the differential intensity of hydro-dynamic tensional stimuli to which the vesicular and cardiac mesenchymal cells, respectively, have been subjected during development.

3. The differential degree of energy possessed by the types of muscle is purely an embryological bio-mechanical problem corresponding to the diverse amounts of optimum tensile work that has been expended in their formation by a dominant extrinsic energetic zone which draws out the pre-muscle mesenchyme in traction between the points of attachment, at least one of which is mobile.

4. The elongation of muscular fasciculi is in the direction of a dominant force extrinsic to the zone of myogenesis just as the strands of a mass of taffy candy are in the direction of the diverging supports, the hands.

5. Muscle tissue is not a self-differentiated product but is a bio-mechanical resultant of an optimum tension. The variable intensity of the optimum tension determines the muscular type. The modifying growing cells receive and respond to the mechanical tensional stimulus. The stimulus, however, is a function of position.

6. In considering the *origins* of the *bladder rhythm* the extrinsic hydro-dynamic tensional stimulus as well as the irritable reacting body—the bladder muscle—is shown to be absolutely necessary as one of the factors accountable for vesicular rhythm.

7. The evidence herein presented proves that the structure of striated muscle is determined by the function it performs and the work it does, and that cross-striated muscle is not formed in anticipation to a future function. The conclusion is warranted that function, in this case, determines structure, and not the reverse.

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CONDITIONS CAUSING AN UNEQUAL DISTRIBUTION OF ERYTHROCYTES IN THE BLOODSTREAM

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The sudden increase of red cells in the animal body under various conditions has lately been investigated by a great number of workers. The general conclusion reached has been that there is a real increase in the number of red cells per unit volume after asphyxia, abdominal massage, administration of adrenalin, etc. The cause of such an increase has been variously ascribed to *a*, decrease in the plasma volume; *b*, formation of new cells; *c*, bringing into the circulation of cells stored away in some interior organ. Only the most recent literature will here be considered.

Lamson (1) who makes use of such factors as adrenalin, fright, asphyxia, etc., to increase the number of red cells, finds that the liver is the regulatory apparatus. He believes that it exercises this function by rapidly eliminating plasma from the blood through its very permeable capillaries, and possibly by liberating cells that have been kept in storage in its tissue spaces. The latter contention seems to be supported by the appearance of a number of smaller cells in the circulation. Lamson found that during experimental polycythemia the average size of the red cell decreases 13.4 per cent. Through determinations of the plasma volume by the Keith-Rowntree-Geraghty method (2) he also found that with the increase in red cells there is a decrease in the plasma volume; not enough, however, to account for the total increase in red cells. It is interesting to note that Lamson gets an increase of red cells after tying, or simply exposing the carotid artery and the jugular vein.

Scott and his co-workers (3) connect the increase in the number of red cells with a rise in blood pressure. They conclude that whenever there is a rise in blood pressure, there is an increase of erythrocytes per cubic meter. Schneider and Havens (4) worked with subjects training

at high and low altitudes. As a general rule they found a marked increase of erythrocytes after muscular exercise both under high and low atmospheric pressure. They believe that there is normally a storage of red cells in the splanchnic area, which in times of stress is brought into the general circulation. One of their reasons for this assumption is that abdominal massage and pressure will cause an increase in the erythrocyte count. Morawitz and Mason (5) working at the height of 3000 meters found that low atmospheric pressure produces an increase of erythrocytes. That this increase is not due to the sudden appearance of young cells in the circulation they prove by determining the amount of oxygen used by the cells themselves; they find this to be approximately the same before and after the increase of the red cells. As young cells consume more oxygen than mature ones, an increase in the number of the former would have been evidenced by an increase in oxygen consumption.

The explanation of the sudden increase in the number of red cells per unit volume as due to an elimination of plasma from the circulation seems, up to the present time, to have the most experimental evidence in its favor. We find little or no experimental support for the theory that cells are brought into the circulation from the blood-forming organs or from some storage-place. The possibility that an equal distribution of red cells could cause the above-mentioned (in such a case only apparent) increases has received some attention, only to be denied, however. It is evident that this is a very important point that must be settled before we can say definitely that adrenalin, asphyxia, etc., cause an increase of red cells per unit volume. Cohnstein and Zuntz (5) have determined the number of red cells per unit volume of blood taken from different parts of the bloodstream under various conditions and state that as far as the number of red cells is concerned, the blood is practically homogenous throughout the circulation. That statement has settled the question for most investigators in this field, who have applied it to conditions in some cases very different from those examined by Cohnstein and Zuntz. Lamson and Scott (1), (2) have made independent determinations, and found that the number of red cells is the same in various parts of the bloodstream. They have not, however, as far as known published any details of those determinations. The evidence for a *uniform* increase throughout the circulation can therefore not be considered very abundant. It was to determine whether there is a uniform increase or not that the first series of these experiments was undertaken.

Methods. Three methods, frequently mentioned in the literature as producing an increase in the number of red cells per unit volume, were used, i.e., adrenalin injected directly into the circulation, asphyxia produced by the administration of illuminating gas, and abdominal pressure caused either by manual manipulation, by placing weights on the abdomen, or by pumping oxygen or air into the peritoneal cavity until the intraperitoneal pressure reached about 10 mm. of mercury.

Eleven experiments were performed, cats being use in every instance. Samples for counting were taken practically simultaneously from the capillary blood and from the blood in the left ventricle. Those two parts of the circulation were chosen because blood could be easily obtained there without disturbing, to any great extent at least, the blood flow. The heart was considered especially suitable to obtain blood from because a cannula can be inserted into it without in the least stopping or retarding the blood. The possibility of causing an artificial increase in the number of erythrocytes through an obstruction was thus eliminated. The capillary blood was drawn from the footpads, the bleeding being caused by a fairly deep cut with a pair of scissors. The drop of blood that appeared was drawn directly into the counting-pipette and diluted in the usual way. The blood from the heart was taken from the left ventricle by means of a syringe and drawn into the counting-pipette directly from the cannula. A Thoma-Zeiss counting chamber was used. Only 80 small squares were counted; no duplicate counts were taken, except in a few cases to determine the limit of error. It was found advantageous to save time in this way, even though allowance had to be made for a greater limit of error. Consequently variations of less than 200,000 have not been taken to indicate with certainty that there has been an increase or a decrease.

Results. The table below contains figures from three typical experiments. The results of all the experiments of this series are quite uniform. Any exception is noted on the following page.

It appears from these figures that the increase of red cells is not uniform throughout the circulation. More cells are found in the capillary blood than in the heart blood after the application of abdominal pressure, and after the administration of adrenalin or illuminating gas. All the experiments of this series gave the same results in this respect. In experiment 2 there is already before the application of the abdominal pressure a great difference in the number of red cells in the capillary blood and in the heart blood. That may possibly be due to fright and struggle during the first part of the anesthesia, a condition known to cause a rise

in the blood count, presumably of the same nature as that caused by adrenalin, asphyxia, etc., i.e., consisting principally in an accumulation of cells in the peripheral blood vessels.

NUMBER OF EXPERIMENT	ANESTHETIC	NUMBER OF RED CELLS PER CUBIC CENTIMETER IN BLOOD FROM		TREATMENT	NUMBER OF RED CELLS PER CUBIC CENTIMETER C : A ½ HOUR AFTER BEGINNING OF TREATMENT, IN BLOOD FROM	
		a. Footpads	b. Heart		a. Footpads	b. Heart
1	Ether	7,190,000	6,690,000	C:a 1 cc. of adrenalin, dilution 1: 10,000 injected into left ventricle of the heart	8,280,000	6,220,000
2	Ether	6,650,000	4,360,000	Pressure applied to abdomen by means of weights c: a ½ hour	7,700,000	4,130,000
3	Ether	6,940,000	7,060,000	Illuminating gas small doses c : a 20 minutes	8,520,000	6,810,000

As already mentioned, statements are frequently found in the literature which are in contradiction to the results of these experiments (Lamson, Scott, et al.). Lamson finds that the same increase of red cells is produced in the heart blood as in the blood of the arteries and veins. In view of the results obtained in these experiments, it must be assumed that Lamson bases his conclusion on an exceptional case. It may be mentioned in this connection that Cannon (6), working on cases of shock, finds a very much higher blood count in the capillary blood than in the veins. This difference he believes due to a stagnation of cells in the capillaries, the main cause of which he believes is an increased friction, brought about by the enlargement of the red cells. He points out that with the lowering of the temperature of the blood there is an increase in the H-ion concentration and that, according to Hamburger, the red cells enlarge as the medium becomes more acid. Scott (2) states that he has taken a great number of blood counts (apparently under normal conditions) from the capillaries of different organs and from the larger vessels simultaneously, and that he always finds the number of red cells per unit volume to be the same in any of those parts of the circulation.

No attempt has been made in these experiments to compare the blood of the arteries and veins with that of the capillaries and of the heart. But the fact that the heart blood in 9 experiments out of 11 shows a decrease in the number of red cells (in two cases a slight increase of about 200,000 was found) after adrenalin, asphyxia and abdominal pressure, indicates that a uniform increase of red cells throughout the circulation cannot be the rule.

It was noted in the above experiments with adrenalin that the greatest increase in number of red cells was found when the temperature of the skin was strikingly lowered through the peripheral vasoconstriction. This effect of the adrenalin apparently does not disappear with the lowering of the blood pressure to normal or below normal level. It is possible that the fall in blood pressure is caused by a vasodilatation in other parts of the body, which leaves the superficial vessels unaffected. Such a possibility is suggested by the findings of Gruber (7) and others that the same dose that causes a vasoconstriction of the vessels of the skin, can cause a vasodilatation of the muscle vessels. The fact that the rise in blood pressure after injection of adrenalin only lasts a few minutes does therefore not necessarily indicate that all effects of adrenalin have worn off in that time. On the contrary, the pale appearance of the skin, the lower temperature of the skin, and the greater difficulty with which bleeding is induced by a skin-cut one half hour or more after an injection of adrenalin into the blood stream, seem to indicate that the effect of adrenalin on the peripheral blood stream has not then yet disappeared. A peripheral vasoconstriction must, according to the temperature observations of Eccles (8), also appear after abdominal massage. After asphyxia a similar peripheral vasoconstriction seems probable, due to stimulation of the adrenals. In any event, asphyxia diminishes the oxygen supply to the tissues. As far as could be determined from the above experiments, therefore, an accumulation of red cells in the peripheral tissues is associated with one or both of the following factors: *a*, a lowering of the surface temperature; *b*, a decrease in the oxygen supply to the tissues.

The next series of experiments was undertaken in order to determine whether the above mentioned factors could be the cause of the peripheral accumulation of red cells, and also at the same time to present a possible explanation of the observed facts.

The effect of lowering the temperature was first examined. It has long been known that a local application of cold has the effect of increasing the red count at the place of application. To determine

whether a cold application could have any effect beyond the parts where the temperature is actually lowered, two experiments were performed in which an area was cooled by means of ice-water, and the blood drawn from a neighboring area sufficiently removed from the cold application not to undergo any appreciable change in temperature. In the experiment cited below, one foot of the cat was placed for 10 minutes in ice-water, so that all the pads were just covered. Before and immediately after the cold application, blood was taken for counting from the skin capillaries just above the knee. The following results were obtained:

NUMBER OF EXPERIMENT	ANESTHETIC	BEFORE TREATMENT		TREATMENT	AFTER TREATMENT	
		Capillary blood count	Heart blood count		Capillary blood count	Heart blood count
1	Urethane by mouth	4,630,000	5,300,000	Foot placed in ice-water	7,020,000	5,310.000

The other experiment gave similar results, except that there was a decided decrease in the number of red cells in the heart blood after the cold application. Both, however, demonstrate that a cold application can cause a rise in the capillary blood count even beyond the area where the temperature is actually lowered. They suggest that some product may be formed under the influence of the cold, which can pass to neighboring areas and there cause cell accumulations in the smaller vessels. A spreading vasoconstriction with an increased friction between the blood cells and the walls of the blood vessels also suggests itself as a possible explanation. There was, however, no sign of such a spreading vasoconstriction. At any rate a decrease in the lumen of the vessels cannot explain why there is an apparent increase in the number of cells in one of the larger blood vessels after adrenalin, for instance, as reported by many (Lamson, et al.). If an increased friction is the cause of an accumulation of cells in the larger vessels (at least when they are momentarily obstructed by a cannula), it does not seem likely that it is the change in lumen that is the cause, but rather some change on the surface of either the blood cells or the endothelial cells, or of both.

It is well known that the tendency of the blood cells to adhere to each other and to the walls of the blood vessels varies under different conditions. Höber (9), Girard (10), Iscovesco (11), et al., have shown that the blood corpuscles in the normal, slightly alkaline blood carry negative charges. The same is true, according to Girard (10), for

solutions of saccharose with Δ = -0.60. If enough organic acid is added to make Δ = -0.64, the corpuscles reverse their negative charges and in an electrical field wander toward the cathode. Mines (12) has obtained the same result with a solution of a trivalent ion such as Ce. He was able to demonstrate that in series of gradually increasing concentrations of $CeCl_3$, the corpuscles in solutions of certain strengths appear to have no charge, and in those of greater strengths assume a positive charge.

It seems beyond doubt in consideration of the work of the above mentioned investigators and of numerous others that in an alkaline medium the blood cells as well as the endothelial cells carry negative charges. It is evident that these charges must be a factor in preventing the cells from adhering to one another and to the walls of the vessels, as bodies with like electrical charges repel each other. A small increase in the H-ion concentration of the medium would tend to cause neutralization of the negative charges of the cells and would therefore diminish a force that tends to keep the cells apart and away from the walls of the vessels. Under such conditions accumulations of cells must form in the smaller vessels, presenting a condition similar to the one observed in stasis, where aggregations of cells can be seen to adhere to the walls of the blood vessels while the plasma is in motion.

To show that solutions of high H-ion concentration will produce a local increase in the number of red cells, experiments were performed in which solutions, such as acid sodium phosphate, were injected subcutaneously. The acid sodium phosphate was prepared by mixing molar H_3PO_4 and molar NaOH in proportions indicated by the tables of Prideaux (13a). Instead of H_2O, normal saline was used for the dilution of the mixture to 100 cc. A series of solutions on both sides of the neutrality point was prepared and tested for hemolysis, and the mixture having the greatest H-ion concentration and not causing hemolysis was used for the following experiment; 0.2 cc. was injected subcutaneously at the root of a foot pad, and blood was drawn from the same foot pad 10 minutes later and also just previous to the injection, and the number of red cells per centimeter determined in both samples of blood. In the same way normal saline and N/50 NaOH were injected into other foot pads, and samples of blood tested before and after the injection. The results are shown in the following table:

NUMBER OF EXPERIMENT	INJECTION FLUID	NUMBER OF RED CELLS	
		a. Before injection.	b. 10 minutes after injection
1	Acid sodium phosphate	6,220,000	8,480,000
2	Normal saline	6,220,000	6,000,000
3	N/50 NaOH	7,320,000	5,660,400

The injection of the normal saline solution was made to ascertain if the injection of a neutral fluid, by pressure or in some other way, could have any effect on the blood count. The above figures indicate that there is practically no effect of an injection of a small amount of normal saline solution. The increase in the number of erythrocytes on one hand and the decrease on the other, after the injections of acid sodium phosphate and N/50 NaOH, suggest that an increase in the H-ion concentration tends to retain the cells in the smaller vessels while an increase in the OH-ion concentration has the opposite effect.

There are two reasons why one should assume an increase in the H-ion concentration in the peripheral tissues after the injection of adrenalin into the blood stream. The blood supply and consequently the oxygen supply to those tissues is diminished as a result of the peripheral vasoconstriction, and a decrease in the oxygen supply is *ceteris paribus* known to be followed by an increase in the H-ion concentration. Furthermore the temperature is lowered in the peripheral tissues which (as well as the blood) contains mixtures of sodium bicarbonate and CO_2, or of sodium hydrogen phosphate and CO_2, the so-called buffer substances; and a lowering of the temperature of those mixtures increases the relative H-ion concentration. Bayliss (13b) states that the alkalinity of a mixture of sodium bicarbonate and CO_2 is approximately four times as great at 38°C. as at 18°C. Those figures also hold true for the blood on account of its having the above mentioned buffer substance in solution.

To test the effect of the temperature on the stability of a suspension of red corpuscles in a mixture if CO_2 and a 1/10 molar solution of sodium bicarbonate in normal saline, the following experiment was performed. Two test tubes were joined together by a communicating tube that opened into each halfway between the open and the closed end so that the whole had the shape of the letter H. This combination of tubes was filled with the above mentioned suspension, and one of the test

tubes entered to half its length into a thermos bottle containing water at 40°C. The other test tube and the connection tube had approximately the room-temperature which was 22°C. Two ordinary test tubes, of approximately the same diameter as the H-tube, were filled with the same suspension, and one inserted half its length into the water of 40°C., and the other kept at room temperature. These last test tubes will be referred to as control tubes. Readings in millimeters were taken of the depth of the clear fluid which was left at the top of the suspensions after the corpuscles had started to sink. The results are tabulated below.

MINUTES FROM THE BEGINNING OF EXPERIMENT	H-TUBE		CONTROL TUBES	
	a. Warm side	b. Cold side	a. Warm side	b. Cold side
	mm.	*mm.*	*mm.*	*mm.*
30	0	14	0	2.5
60	0	24	0	3.5
90	0	34	0	4.5
120	0	44	0	5.5

According to these figures there is no evidence of any sedimentation when the temperature is that of the normal body. That this absence of sedimentation is not due to convection currents, which may be thought to "stir" the warm suspensions, but to the nature of the solution is made evident by the fact that if blood is drawn into a 0.9 per cent solution of NaCl in proportion 1 to 10, and the above procedure repeated, the more rapid sedimentation will take place on the warm side. The most plausible explanation of the more rapid sedimentation in the cold sodium bicarbonate-CO_2 mixture is therefore to be sought, not in the absence of convection currents, but in the greater H-ion concentration of the cold suspension, being three to four times as great as of the warm.

That the positive ions, particularly the H-ions and the trivalent ones, increase up to a certain degree of concentration the rate of sedimentation in the case of a suspension of red corpuscles, is shown by the work of Mines (12), Linzenmeier (14), Girard (10), et al. The explanation given is that the negative surface charges of the corpuscles are neutralized, and that under those conditions cell aggregates are formed, many times the size of the individual corpuscle. This diminishes the stability of the suspension because of two suspensions, the one with the larger particles is less stable, other things being equal. This explains the difference in stability between corpuscular suspension in cold and in warm

sodium bicarbonate-CO_2 mixtures, the former having a greater number of H-ions which tend to neutralize the negative surface charges of the corpuscles and thus bring about a more rapid sedimentation.

It was observed in the above experiment that the sedimentation in the cold side of the H-tube is many times greater than in the cold control tube. This can probably be explained by a migration of positive ions from the warm to the cold side of the H-tube. It is well known that a difference in potential appears when two solutions of unequal strength come in contact. Except in the case we deal with solutions of infinite dilution, the degree of ionization is the most important factor. The greater the temperature coefficient of dissociation, the greater is the difference in the number of ions found on the warm and on the cold side. It seems self-evident that under those conditions the ions will start to migrate toward the side that has the smaller number of ions, and the positive H-ions, having the greatest velocity of all, will outstrip the rest. This migration alone will therefore tend to make the relative H-ion concentration greater on the side that has the fewer number of ions, in this case on the cold side. This may, however, not be the whole explanation. When working with corpuscular suspensions in other solutions, for instance of sodium sulphate or sodium chloride, it was seen that both the warm and the cold side of the H-tube had an increased rate of sedimentation compared with the control tubes. It is possible that during the migration that takes place in the H-tube, the distance between the positive and the negative ions becomes greater than in a solution of uniform temperature, and that the positive ions under those conditions more readily neutralize negative bodies with which they come in contact.

From the above experiments one can draw the conclusion that in the blood, containing as it does a mixture of sodium bicarbonate and CO_2, the corpuscles more readily aggregate when the temperature of the blood is brought down; and also that if the blood of decreasing temperature is in communication with blood of higher temperature, the effect of the cold (causing an aggregation of corpuscles) is accentuated. Such a condition may account for the fact that one normally finds the red blood count somewhat higher in the superficial capillaries than in the larger vessels. It may also account for cases such as the capillary accumulation of red cells in shock (Cannon) where the blood apparently flows slowly enough through the peripheral capillaries to become more or less cooled off. But after the administration of adrenalin or after abdominal pressure the temperature of the blood itself

probably undergoes only a small change, if any. On the other hand the temperature of the skin and directly underlying tissues must go down, as already observed. Under those conditions there will be a higher H-ion concentration in the periphery of the body than in the interior. This effect is increased by the diminution of the oxygen supply to the skin and underlying tissues, due to the decrease in the capillary bed. A migration of H-ions will in such case take place from the surrounding tissues into the peripheral blood stream, and there bring about the effect shown for H-ions and other positive ions, especially the trivalent ones, i.e., cause an aggregation of the blood corpuscles. At the same time the endothelial cells of the walls of the blood vessels will repel the corpuscles less than normally. It is easy to understand how under those conditions the blood cells tend to accumulate in the capillaries. An increase of corpuscles may for the same reason appear in the larger vessels, especially with a slight obstruction such as that produced when a small cannula is inserted into the vessel. It is evident that under normal conditions, when the tendency of the cells to adhere to one another is less, an obstruction will not cause the same amount of retention of cells as in the above described conditions.

The question next arises whether the enlargement of cells or the elimination of fluid from the blood stream could be contributing factors in the unequal distribution of the cells. As already mentioned, Lamson found a decrease in the corpuscular volume in experimental polycythemia. He thinks that this indicates that new cells are brought into the circulation. In view of the fact that the cells readily change in size under varying conditions such an interpretation does not seem well founded. According to Hamburger (15) the red cells are larger in the jugular vein than in the carotid artery. He ascribes the difference to the greater CO_2 content of the jugular blood. At the same time, however, he shows an increased alkalinity of the jugular blood (16). It cannot therefore be an increase in the H-ion concentration that causes the enlargement of the cells (as Cannon (6) seems to conclude). Nor would we expect an increase in the H-ion concentration to cause an enlargement as long as the blood remains on the alkaline side (which, of course, always is the case except under extreme conditions). A disappearence of negative electrolytes from the surface of the cells will decrease the outward stress between the negative ions on the surface of the cell and the corresponding positive ions present in the plasma. It is evident that the smaller such a stress, the greater will be the surface tension and the smaller the size of the cell. We must therefore leave out the

enlargement of corpuscles as a factor in causing stagnation of the blood in the capillaries.

The passing of fluid from the blood to the tissues has been much emphasized in recent literature. Quantitative measurements of the amount that leaves the blood stream have been made. It does not seem impossible that a sudden diminution of plasma could cause a stagnation of blood cells in the peripheral capillaries if the elimination of plasma took place there. However, according to Lamson (1) et al., it takes place almost exclusively in the liver. With the present evidence there is therefore no reason to suppose that plasma elimination plays any rôle in the unequal distribution of the red cells.

Doubt is thrown on accuracy of the published (1) measurements of the plasma eliminated from the bloodstream after the injection of adrenalin, etc., when we consider that those measurements indicate an elimination of plasma almost large enough, in many cases at least, to account for a general increase of red cells throughout the circulation. The results of heart blood counts, described above, show that there is as a rule no *general* increase. The accuracy of the Keith-Rowntree-Geraghty method of plasma volume determination is also questioned by Harris (17). His main objection to the method is that, if one for instance takes the readings after 2½ minutes after the injection of the stain, this is not yet well distributed in the bloodstream, while after 4 minutes some of the stain has already disappeared into the tissues. This criticism, however, does not imply that the method could not give good *relative* values if the conditions, under which the determinations are made remain unchanged. But with an increase in the H-ion concentration, as for instance after asphyxia, the stain probably disappears faster from the bloodstream than before. Azada (18) has shown that in acidosis the tissues take up the stain much more readily than otherwise. Another source of error in connection with this method is that the cells themselves with changing H-ion concentration may eliminate or take up water. This would of course change the plasma volume even if no plasma passed in or out of the blood vessels. It is therefore difficult to determine to what extent an elimination of plasma from the blood stream takes place under those conditions.

Lamson states that no increase follows the administration of adrenalin if the liver is excluded from the circulation. Believing as he does in the plasma elimination as the main cause of the "increase" of red cells after adrenalin, he concludes that practically all the elimination takes place in the liver. It is, however, possible to explain the effect of the

exclusion of the liver from the circulation, even if we assume that the administration of adrenalin is followed principally by an unequal distribution of the red cells, rather than by a general increase due to a disappearance of plasma from the bloodstream.

In discussing the effect of adrenalin, the importance of lowering of the temperature in the peripheral tissues for the increase of the H-ion concentration there, and thereby for the retention of blood cells in the peripheral blood vessels, was emphasized. It was also pointed out that the blood and oxygen supply to the peripheral tissues may be diminished while it may be increased to other parts. There must under those conditions be an increase in the H-ion concentration confined more or less exclusively to the peripheral tissues. If the H-ion concentration increased uniformly throughout the body, we would not expect to see any marked effect on the blood count because with a uniform increase, *a*, there would be no migration of ions, which has been shown to have an effect on the formation of cell aggregates; and *b*, the cells would not accumulate in the superficial more than in the internal capillaries, and the number of cells that were retained in the former could not be so great as if the superficial circulation alone were concerned. Thus, if the temperature of the body is lowered uniformly, and with that an increase in the H-ion concentration takes place in the interior as well as in the exterior, no marked accumulation of red cells in any one part of the body is to be expected. This is probably what takes place when the abdomen is opened and the liver, the warmest organ of the body, is eliminated from the circulation. Lamson's statement that no increase follows the administration of adrenalin after the elimination of the liver from the circulation can be explained, therefore, even if one assumes that the effect of adrenalin is not so much a general concentration of the blood as an accumulation of the corpuscular elements in the peripheral bloodstream, and an increased tendency of the cells to form aggregations.

SUMMARY

1. After adrenalin, abdominal pressure or asphyxia there is an increase in the peripheral vessels of erythrocytes per centimeter as shown by many investigators. This increase is not general, however, and in most cases it is accompanied by a decrease in the number of cells in the heart blood.

2. If red corpuscles are suspended in a mixture of 1/10 molar sodium bicarbonate and CO_2 in normal saline solution, the suspension is stable if the temperature is kept at about 38°C. It becomes unstable at a

lower temperature, i.e., the corpuscles begin to sink. This is associated with the relatively greater H-ion concentration which exists at a lower temperature, and which is supposed to neutralize or diminish the negative charges on the surface of the cells and thus prepare the way for the formation of cell-aggregates, which sink more readily than the free corpuscles.

3. The effect of the low temperature, making the suspension of corpuscles in the sodium bicarbonate-CO_2 mixture unstable, is more marked if the cold suspension is in connection with a warm suspension. The explanation suggested is that during the migration of ions that takes place when two solutions with an unequal number of ions come in contact, the positive ions become separated from the negative ions and under those conditions more readily neutralize the negative surface charges of the red cells, and also that more H-ions than OH-ions will reach the cold side (from the warm) on account of the greater migration velocity of the former, thus relatively increasing the H-ion concentration on the cold side.

4. A local increase in the H-ion concentration, brought about by a subcutaneous injection of a dilute acid solution (acid sodium phosphate), is followed by an increase in the number of erythrocytes per centimeter in that part.

5. It is pointed out that there are good reasons for assuming an increase in the H-ion concentration in the peripheral tissues after the administration of adrenalin, asphyxia and abdominal pressure. Such an increase in the H-ion concentration would explain the peripheral accumulation of red cells, as it would cause the red cells to lose, partly at least, their negative surface charges, which tend to prevent the cells from adhering to one another and to the walls of the vessels.

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